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PHYSICO-CHEMICAL STUDIES ON PROTEINS. VII. THE PEPTIZATION OF GLIADIN BY SOLUTIONS OF INORGANIC SALTS [1]

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Introduction and Historical

In earlier papers Gortner, Hoffman, and Sinclair (1928, 1928a, 1929) demonstrated that the protein complex of wheat flour could be peptized to different degrees when treated with inorganic salt solutions, and that a marked lyotropic series of anion effects was evident. Thus the averages of the data for twelve representative samples of wheat flours treated with N/1 salt solution showed that KF peptized 13%, KCl 23%, KBr 37%, and KI 64% of the total nitrogen. The amounts of nitrogen peptized by the bromide and iodide solutions were far in excess of the amounts which could be assigned to the quantities of "albumin" and "globulin" known to be constituents of the wheat flour, and it therefore became apparent that one or both of the proteins of the "gluten" complex was being peptized and rendered "salt soluble" by the action of the salt solution. Preliminary experiments indicated that this peptizable protein was gliadin, and accordingly a series of experiments was planned in which the peptization behavior of pure gliadin was studied and in which attempts were made to fractionate gliadin as a test of the chemical homogeneity of the protein.

In our earlier papers (Gortner, Hoffman, and Sinclair (1928, 1929) and Staker and Gortner (1931)) most of the literature dealing with protein "solubility" has been discussed. It was pointed out that the term "solubility" in the case of "proteins" should probably be replaced by the term "peptization" or "peptizability," since, in the case of the proteins, we are dealing with colloidal substances and that these,

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in general, form colloidal sols rather than true solutions. It is generally recognized that colloidal micelles in different degrees of dispersion can be present in a sol and it was accepted as a working hypothesis that the "solution" effect of the different inorganic salt solutions on the wheat flour protein complex reflected the interfacial actions of the protein complex, the water, and the specific ions which were present in the system.

Simultaneously with our studies, Haugaard and Johnson (1930) were carrying out studies in the Carlsberg Laboratory in an attempt to fractionate gliadin and thus decide whether or not gliadin is a definite chemical entity. These authors reach conclusions which are somewhat at variance with our own, accordingly their findings will be again referred to in a later section of this paper.

Parallel with the studies reported in the present paper, Staker and Gortner (1931) were studying the peptization behavior of twenty-eight seeds and grains, including eight of wheat (*Triticum*), barley, and oats, six legumes, three crucifers, corn, millet, sorghum, teosinte, Brazil-nuts, hemp, and sunflower. The salts used were N/2 solutions of KF, KCl, KBr, KI and K_2SO_4. A marked lyotropic series was observed for all seeds with the exception of the "corn group," including maize, millet, sorghum and teosinte, and the Brazil-nut. These five seeds showed an almost constant peptization of the protein complex in all of the salt solutions which were tested. The range of the peptizability of the seed proteins in the different salt solutions was, however, very great. Thus in N/2 KF solutions the range in percentage of the total nitrogen peptized was from 6.22% (teosinte) to 85.23% (field peas); for N/2 KCl from 6.39% (teosinte) to 86.09% (peanut); for N/2 KBr from 7.05% (teosinte) to 89.51% (peanut); for N/2 KI from 7.69% (teosinte) to 95.18% (field peas) and for N/2 K_2SO_4 from 6.42% (teosinte) to 89.19% (peanut). Similarly for certain seed meals the range of peptizability of the protein complex showed wide ranges, as for example hempseed meal where the values are KF 24.65%, KCl 30.32%, KBr 57.42%, and KI 70.58%, and for the average of the *Triticum* species KF 24.86%, KCl 30.98%, KBr 40.16%, KI 55.01% and K_2SO_4 26.24%. Staker and Gortner conclude their paper with the statement that protein "solubility" is in reality protein peptization, and that the technic which is employed may largely determine the yield and the properties of the protein complex which is isolated. The existence of true plant "albumins" as definitely characterized chemical entities is questioned. The available evidence indicates that many of the preparations which have hitherto been regarded as homogeneous and distinct plant proteins may in reality either be heterogeneous mixtures or else merely fragments of a larger and more complex protein "micelle."

Experimental

The Problem

In an earlier paper of Gortner, Hoffman, and Sinclair (1928) occurs the following statement:

"Our experiments would indicate that all of the various protein fractions (with the exception of the proteose) which have been considered fixed entities, and characteristic of wheat flours are, if we follow the prescribed routine procedure for protein isolation, capable of realization, but that, if the routine procedure is somewhat altered, fractions having different properties and characteristics are obtained. Thus, for example, a normal solution of KBr extracts a given percentage of protein from a wheat flour which is far in excess of the amount of protein extracted by a normal solution of KCl. If now the KBr-protein solution is subjected to dialysis, so as to remove the salt, a precipitate of protein is formed as one would expect in dealing with a solution of a 'globulin' and *this precipitate is now completely soluble in normal KCl solution.* In the flour, part of the protein was not extractable by KCl solutions, but following a KBr extraction it is peptizable by KCl. Unfortunately, far too little is known regarding the factors involved in the peptization of a protein to enable us to assign a cause of these observed facts. The cause is probably complex and will be found to involve the nature of amino acid linkages, secondary valences and polar groups as well as degree of hydration and specific ionic effects."

This statement raised the following questions toward the solution of which the present data are directed.

1. What would be the relative effect of neutral salt solutions on a pure protein?

2. Would it be possible to peptize a definite quantity of a pure protein by neutral salt solutions?

3. If there occurred a "peptizable" and a "non-peptizable" fraction, would these fractions show any difference in chemical constitution?

4. Are there any differences in the physical properties of the various fractions of protein prepared by fractional peptization?

The Material

Since gliadin appeared to be the gluten protein chiefly responsible for the larger quantities of nitrogen found in the KBr and KI solutions, and since gliadin can be readily prepared in quantity by more or less standardized procedure, it was selected as the protein for the present study. Four different preparations of gliadin were made utilizing (*a*) the standard "70% alcohol method," which is essentially that proposed by Osborne (1907), (*b*) the "acetic acid method" of Blish and Sandstedt (1926), (*c*) gliadin prepared by the "alcohol method" and later dissolved in 0.07 N acetic acid and "repurified" by the "acetic acid method," and (*d*) gliadin prepared by the "acetic acid method" and further purified by electrodialysis.

Methods

Because of the later findings as to importance of the method of preparation, the exact methods which we used are given in detail:

The Preparation of Gliadin ("A") by the Alcohol Method. A strong wheat flour was made into a stiff dough by means of a laboratory dough mixer. After allowing the dough to stand under water for one or two hours in order to form the gluten, it was washed, in a stream of tap water, as free as possible from starch. The dough was then minced in a meat chopper. The hydrated gluten was then extracted with 70% ethyl alcohol, sufficient stronger alcohol being added to yield this concentration, assuming that the moist gluten had an initial moisture content of 60%. The gluten residue was extracted twice more with 70% ethyl alcohol. All extractions were made at room temperature, heating being avoided in order to prevent any possible denaturization of the protein. The alcoholic filtrates were then filtered through paper pulp yielding a clear yellow liquid. This large volume of extract was reduced *in vacuo* at a low temperature (50° C.) until the gliadin precipitated on the bottom of the flask. The gliadin mass was then dissolved in a minimal quantity of 70% ethyl alcohol. Upon pouring this alcoholic solution of the gliadin into a large volume of cold water, the gliadin precipitated in a mass which could be collected around the stirring rod. If precipitation did not occur immediately, a little LiCl or NaCl was added to produce complete precipitation. This precipitate was dissolved in as small a quantity as possible of 70% alcohol, and the water precipitation was repeated. A final dispersion in 70% alcohol was made and the gliadin was precipitated by pouring into a mixture of absolute alcohol and ether containing a trace of electrolyte such as LiCl. The material so obtained was allowed to stand three or four hours in absolute alcohol, filtered on a Buchner funnel and finally dried in an oven at 50° C. The gliadin was obtained in the form of a white brittle mass which could, in a very short time, be ground to a fine white powder in a ball mill.

Preparation of Gliadin ("B") by the Acetic Acid Method. Blish and Sandstedt (1926) devised this method and claim that this procedure yields a product of superior quality. The experimental details of the method as described by Blish and Sandstedt were rigidly followed in every detail in the preparation of our "Gliadin B."

Gliadin "C." This sample of gliadin was made from gliadin which had originally been prepared by the alcohol method. The gliadin was dissolved in 0.07 N acetic acid and carried through the identical process as described by Blish and Sandstedt (1926) in the preparation of gliadin by the acetic acid method, i.e., precipitated from the acid solution with

LiCl, dissolved in alcohol and reprecipitated several times from a mixture of absolute alcohol and ether, finally washed with absolute alcohol and dried in oven at 50° C. The product obtained was a brittle white mass, which was easily ground to a fine powder in a porcelain ball mill.

The Preparation of Electro-dialyzed Gliadin ("D"). A sample of the gliadin prepared by the acetic acid method was "dissolved" in distilled water. A surprising amount passed into "solution." It was possible to disperse as much as 16 gms. of the "acetic acid" gliadin in 100 cc. of water. This aqueous "solution" of the gliadin was then electro-dialyzed between platinum electrodes, using a three-compartment dialyzing box similar to that figured by Gortner (1929) or Morrow (1927). The compartments were separated by cellophane membranes, and sheet platinum was used for the electrodes. The protein sol was placed in the center compartment, and the two platinum electrodes, which were placed in the end compartments respectively, were connected to a source of direct current. An ammeter and a resistance were placed in the circuit. As electrolysis proceeded the cathode compartment became alkaline and the anode compartment acid. The final solution taken from the anode compartment showed a pH 2.67 (after dialysis was completed). During electrolysis the protein precipitated in the center compartment, and when the amperage had decreased to zero, the supernatant liquid in the middle compartment showed a pH 4.85. This supernatant liquid was then decanted, the precipitated protein was dissolved in 70% alcohol and poured into a mixture of absolute alcohol and ether. It was afterwards washed with absolute alcohol and dried at 50° C.

The volume of the anode and cathode liquids were separately reduced *in vacuo* and the solutions later made up to 250 cc. volume. A Kjeldahl determination on both of the liquors showed the absence of nitrogen, demonstrating that no protein or residues of partially or completely hydrolyzed protein were transferred during the electrodialyzing process. The liquor from the anode compartment gave a positive chloride test with silver nitrate. The liquor from the cathode compartment reacted slightly alkaline. Ash determinations on the gliadin before electrodialysis indicated 0.05% and after electrodialysis 0.07% ash.

Comparison of the Properties of Gliadin Preparations. A comparison of the properties of gliadin preparations prepared by the 70% alcohol and acetic acid procedures showed marked differences in regard to several fundamental properties. It is needless to emphasize that in applying two different methods to the preparation of a colloid of biological origin, there is no certainty of obtaining an identical product, at least in so far as its physico-chemical properties are concerned. One

of the most distinguishing characteristics of the two samples of gliadin is their difference in "solubility." The gliadin prepared by the 70% alcohol method does not possess the "solubility" in any solvent, that is possessed by gliadin when prepared by the acetic acid modification.

Gliadin prepared by the established alcohol method is supposedly "soluble" in 70% alcohol. No doubt the degree of "solubility" depends upon several factors such as temperature treatment during the extracting process, and the denaturing effect of alcohol upon the gliadin. Such denaturization effects have been shown by Dill and Alsberg (1925) to take place in 70% to 80% alcohol. Gliadin prepared by the 70% alcohol method is more or less difficultly "soluble" in 70% ethyl alcohol after it has once been completely dehydrated. In fact it is rarely possible to completely "dissolve" 1 gm. of dry gliadin in 100 cc. of 70% alcohol in the cold. When such a "solution" is heated, more passes into "solution," but, on cooling, a part of the gliadin again separates. Even prolonged standing in contact with 70% alcohol does not appreciably aid "solubility." In general the "solution," unless filtered, remains decidedly turbid.

The situation is quite different in regard to the "solubility" of gliadin prepared by the acetic acid method. This material is extremely "soluble" in 70% alcohol, and furthermore, the "solution" is practically free of turbidity. The gliadin "dissolves" immediately in the alcohol, and on long standing no trace of turbidity results.

It is a well established fact that ordinary gliadin is only slightly soluble in water. This low solubility of gliadin in water is quite significant when compared to the solubility of gliadin prepared by the acetic acid method. This latter is extremely soluble in distilled water. During 36 hours in a "shaking-machine," 16 gms. of this gliadin can be readily dispersed in 100 cc. of distilled water; in addition, if more gliadin is added, a little at a time, and if the solution is shaken vigorously, still more will be peptized. The solution becomes very viscous, and has somewhat of an amber color, but it possesses very little turbidity. No gliadin precipitates from such a solution on long standing. It would be very difficult to determine the exact solubility of this material in water, since the hydration capacity is so great that there is no sharp line of solubility, as in the case of a crystalloid. Just as long as there is enough free water in the solution, some gliadin will be dispersed. Evidently, there must be a concentration at which very little free water will remain and then gliadin would cease to be dispersed, but this concentration is not easy to determine experimentally. The process is one of a gradual taking up of water and finally, when all the water is adsorbed, a viscous gel results.

This great difference in solubility of the two gliadin preparations is

very important and significant in its bearings on protein preparation methods. It is evident that there must be some cause for such an extreme difference in solubility of two substances, which are supposedly the same substance chemically. The only differences in the two methods of preparation are, (1) the solvent used for the extraction of the initial gluten, and (2) the method of precipitation, which requires the use of a neutral salt, in this instance LiCl. Table I shows certain comparative analyses of the two preparations. The acetic acid gliadin certainly does not owe its high solubility to the presence of electrolytes that remain when the sample is ashed. A spectroscopic examination of the "soluble" gliadin showed the presence of lithium, but apparently very small amounts were present. Another possibility that may account for the high degree of peptizability is the adsorption of acetic acid from the solution. The pH of an aqueous sol of the two gliadins is widely different and indicates that the adsorption of traces of acetic acid probably is responsible for the different "solubility" behavior of the preparations.

This assumption is confirmed by the data in Table I showing the titratable acidity of the various samples using Foreman's (1920) method of titrating in 80% alcohol with phenolphthalein as an indicator. This technic has been used by Woodman (1922) and by Hoffman and Gortner (1925) to determine the content of free carboxyl groups in proteins. Gliadin prepared by the alcohol method required 5.7 mgs. of NaOH to neutralize the free carboxyl groups of the native protein, whereas the

TABLE I

COMPARATIVE ANALYSIS OF THE VARIOUS GLIADIN PREPARATIONS

	Gliadin						
	A	B	C	D	E	F	G
Ash %	0.30	0.05		0.07	0.10	0.26	
Cl (mgs. per gm.)	1.2	4.2		0			
Phosphorus	trace	trace		trace			
pH of aqueous "solution"	6.46	3.99		6.50	6.40	6.45	6.50
Mgs. NaOH required to neutralize 1 gm. (in 80% alcohol) to phenolphthalein	5.71	14.86	15.42	5.14	5.14	4.57	4.57
Total N %	17.65	17.35	17.45	17.50	17.45	17.50	17.48
True amide N—% of total N	25.16	25.66		24.99	24.80	25.47	25.50
Free amino N—% of total N	2.18	2.15		2.12	2.11	2.11	2.08
Total sulphur	0.58	0.78	0.66		0.62		
Hausmann nitrogen distribution							
NH_3–N	25.50	25.28			25.48	25.30	25.81
Humin N	0.81	0.90			0.71	0.74	.95
Basic N	10.49	10.50			10.50	10.99	10.85
Non-basic N	63.92	63.82			63.54	63.37	63.50
% recovery	100.72	100.80			100.23	100.40	100.14

same gliadin " repurified " through the acetic acid process required 15.4 mgs. of NaOH which was essentially the amount (14.9 mgs.) required to titrate the gliadin originally prepared by the acetic acid process. Furthermore, when the acetic acid gliadin was electrodialyzed the NaOH requirement fell to 5.14 mgs. Taking 5.14 mgs. NaOH as representing the free carboxyl group requirement of the molecule, we find that the gliadins prepared by the acetic acid method contain respectively (gliadin " B ") 14.57 mgs., and (gliadin " C ") 15.43 mgs. of acetic acid per gram of protein. This acetic acid was exceedingly firmly bound (adsorbed?). It could be removed only by electrodialysis. Repeated solution of the gliadin in 70% alcohol followed by reprecipitation with absolute alcohol and ether, or precipitation by pouring the alcoholic solution into a large volume of water and subsequent dissolving in alcohol followed by the absolute alcohol and ether treatment, failed to remove it from the protein. This amount of adsorbed (?) organic acid, however, was sufficient to alter the apparent " solubility " of the protein in water from a normal value of 0.03 to 0.09 gm. per 100 cc. to a value in excess of 16.0 gms. per 100 cc. of solvent.

The Preparation of Gliadin " E " (KI soluble) and Gliadin " F " (KI insoluble). 100 gms. of gliadin " A " (alcohol method) was treated with one liter of molar KI solution for three consecutive times, making a total volume of three liters of molar KI solution per 100 gms. of gliadin. The first liter of salt solution was allowed to stand in contact with the gliadin for several hours with vigorous shaking at intervals. After the non-peptized protein had settled, the supernatant liquid was decanted, and another liter of salt solution was added to the non-peptized residue. This system was shaken in a shaking machine for twelve hours and then allowed to stand for several hours until the residual protein settled on the bottom of the flask. The supernatant liquid was decanted, and the non-peptized protein was given a third treatment with a liter of molar KI solution similar to the second treatment. The three extractions were combined and passed through a Sharples Super-Centrifuge in order to remove any finely divided non-peptized protein particles. The liquid so obtained appeared slightly turbid, but further centrifuging failed to completely clear the solution. The centrifuged liquid was then dialyzed in a cellophane bag against distilled water until free of potassium iodide. The gliadin precipitated on the bottom of the bag, leaving the supernatant liquid with only a slight turbidity. The precipitated protein was next dissolved in as small a volume as possible of 70% ethyl alcohol and allowed to stand over night. The gliadin was precipitated from the 70% alcohol solution by pouring into a mixture of absolute alcohol and ether. A very small trace of electrolyte, such as LiCl, aided in the precipitation. The protein precipitated in fine flakes, later

gathering into threads. The protein was filtered on a Buchner funnel and allowed to stand in absolute alcohol overnight. It was then filtered and the white mass was dried at 50° C. The amount of protein obtained was 60 gms. Some of the salt-soluble protein was lost during the process of purification. This sample was designated the "*potassium iodide soluble fraction.*"

The non-peptized residue that remained after extracting with molar KI was dissolved in as small a quantity as possible of 70% ethyl alcohol and the solution concentrated *in vacuo* to a syrup. The protein was precipitated by pouring this syrup into a large volume of distilled water, this procedure being adopted in order to free the protein from any potassium iodide left in the residue. In this state of hydration some of the protein dispersed. The precipitated protein was washed several times with water, redissolved in 70% alcohol and reprecipitated from a mixture of absolute alcohol and ether, and washed with absolute alcohol. It was then filtered, and dried at 50° C. The final product, ground in a ball mill, formed a fine white powder. This material was designated as the "*potassium iodide insoluble fraction.*"

The Preparation of Gliadin "G" (KBr Insoluble). The treatment of gliadin with molar KBr solution was performed in the same manner as the peptization of gliadin with molar KI solution. Since the amount of gliadin ("A") peptized by molar KBr solution is much less than the amount peptized by molar KI solution, and since it would require an enormous volume of molar KBr solution to obtain enough of the *KBr soluble fraction* for experimental work, it was decided to extract gliadin ("A") with molar KBr solution in a manner as described above, and then use only the KBr non-peptizable residue in the experiments which follow.

The purification of the "KBr insoluble fraction" (gliadin "G") was carried out by the same technics that were employed in the purification of the "KI insoluble" residue. The final dry white powder was designated as the *KBr insoluble fraction.*

The Peptization of Gliadin by Neutral Salts. The data on nitrogen peptized are recorded in Table II. In Table III are recorded data of the nitrogen peptized by an initial extraction with KBr and KCl solutions of different molar concentrations. All of the extractions indicated in Tables II and III with the salt solutions were practically water clear. In a few cases there occurred some turbidity, but these solutions could be freed from turbidity by filtering.

The values of Table II were obtained by the following procedure: Gliadin was added to the salt solution in a ratio of one part of protein to 100 parts of the salt solution, and the mixture was shaken vigorously at intervals for 24 hours. The solution was allowed to stand for an

TABLE II

THE MILLIGRAMS OF NITROGEN AND THE PERCENTAGE OF GLIADIN PEPTIZED BY VARIOUS SALT SOLUTIONS

Material	Initial amount of gliadin	Salt solution used	Amount salt solution used in each extraction	Extractions				
				No. 1	No. 2	No. 3	No. 4	Total
	Gms.		Cc.					
Gliadin "A" (alcohol method)								
Mgs. N peptized	3	M KI	300	283.20	91.80	13.20	13.20	401.4
% gliadin peptized				53.48	17.33	2.49	2.49	75.79
Gliadin "F" (KI insoluble)								
Mgs. N peptized	1	M KI	100	36.00	12.8	4.40	4.20	57.40
% gliadin peptized				20.57	7.31	2.51	2.40	32.79
Gliadin "E" (KI soluble)								
Mgs. N peptized	3	M KI	300	400.2	40.2	9.2	5.4	455.0
% gliadin peptized				76.44	7.67	1.75	1.31	87.17
Gliadin "G" (KBr insoluble)								
Mgs. N peptized	5	M KI	500	504.00	95.00	28.00	19.00	646.0
% gliadin peptized				60.39	11.38	3.35	2.27	77.39
Gliadin "A" (alcohol method)								
Mgs. N peptized	5	M KBr	500	111.00	54.50	43.50	22.00	231.00
% gliadin peptized				13.91	6.83	5.45	2.75	28.94
Gliadin "G" (KBr insoluble)								
Mgs. N peptized	5	M KBr	500	79.00	52.60	24.00	18.40	174.00
% gliadin peptized				9.46	6.30	2.87	2.20	20.84
Gliadin "E" (KI soluble)								
Mgs. N peptized	5	M KBr	500	102.00	63.00	49.00	27.00	241.00
% gliadin peptized				12.54	7.70	5.99	3.32	29.55
Gliadin "F" (KI insoluble)								
Mgs. N peptized	1	M KBr	100	10.20	7.40	3.40	4.00	25.00
% gliadin peptized				5.82	4.22	1.94	2.28	14.26
Gliadin "A" (alcohol method)								
Mgs. N peptized	5	M KCl	500	20.00	11.00	9.00	6.00	46.00
% gliadin peptized				2.50	1.37	1.12	0.75	5.74
Gliadin "E" (KI soluble)								
Mgs. N peptized	5	M KCl	500	12.00	8.50	5.00	3.55	29.05
% gliadin peptized				1.46	1.03	0.61	0.42	3.52

additional 24 hours and then centrifuged. The supernatant liquid was designated as *extraction No. 1.* The non-peptized protein residue from extraction No. 1 was treated in the same manner with a second similar volume of salt solution, the peptized portion being designated as *extrac-*

TABLE III

THE PEPTIZATION OF GLIADIN "A" BY VARIOUS CONCENTRATIONS OF KBR AND KCL SOLUTIONS

	Intial amount of gliadin	Salt used	Amount salt solution	Molarity of salt solution			
				0.25	0.5	1.0	2.0
	Gms.		Cc.				
Gliadin "A"							
Mgs. N peptized	3	KBr	200	45.5	56.2	35.7	35.7
% gliadin peptized				9.50	11.74	7.46	7.46
Gliadin "A"							
Mgs. N peptized	3	KCl	200	22.9	16.2	8.4	6.5
% gliadin peptized				4.78	3.38	1.75	1.35

tion No. 2. The non-peptized residue from the second treatment was treated with a third similar portion of fresh salt solution to secure *extraction No. 3,* and a fourth treatment yielded *extraction No. 4.* An appreciable quantity of protein " insoluble " in the salt solution remained as the residue from these 4 extractions. Kjeldahl nitrogen determinations were made on the extractions Nos. 1 to 4. The milligrams of nitrogen peptized, and the percentage of gliadin extracted, expressed as the percentage of total nitrogen, are recorded. In Table II it will be seen that, in general, the first extraction peptized the largest amount of gliadin. The second extraction peptized an appreciable quantity, while in the third and fourth extractions a comparatively small amount was peptized.

An important factor to be considered was whether or not the purification of the " insoluble residue " would render them capable of being further peptized by normal solutions of KBr and KI and whether the purification of the " soluble fraction " would result in a product which would leave an " insoluble residue " on a second treatment. This point was tested by running extraction experiments on the various " purified " fractions, the results of which experiments are recorded in Table II.

Discussion

The Chemical Analysis of the Gliadin Fractions. The analyses of the various gliadin fractions are recorded in Table I.

The Blish-Sandstedt (1926) method yields gliadin (" B ") of lower ash content than does the original alcohol method (gliadin " A "). They report gliadin with an ash content of 0.12%. Our gliadin obtained by their method had an ash content of 0.05%. This preparation was purified several times by reprecipitation from a mixture of alcohol and ether. Gliadin " A " prepared by the alcohol method yields an ash content of 0.30%. This preparation also was precipitated twice from water and once from an alcohol-ether mixture. The samples of gliadin prepared by the two methods seem to be of equal quality as judged by appearance such as color, texture, etc. However, the hydrogen-ion concentration of the two gliadins in water (1 gm. in 100 cc.), showed that the pH of the gliadin " B " solution, prepared by the acetic acid method, was 3.99, while the pH of the gliadin " A " solution prepared by the alcohol method was 6.46. The pH values for gliadins " D," " E," " F," and " G " are essentially identical with that for gliadin " A " and are in the range which has been regarded as characteristic of gliadin. Gliadin prepared by the acetic acid method is therefore contaminated with acetic acid, and the Blish and Sandstedt method cannot be used for preparing gliadin suitable for physico-chemical studies unless the product is subsequently electrodialyzed.

Foreman (1920) demonstrated that amino acids in 85% alcohol can be titrated directly with alkali using phenolphthalein as indicator. Woodman (1922) found gliadin to require 19.7×10^{-5} gram equivalents of NaOH per gram of protein. Hoffman and Gortner (1925) found that one gram of gliadin required 18.86×10^{-5} gram equivalents of NaOH for neutralization. The same method was employed in the present study. In Table I are recorded the milligrams of sodium hydroxide required to neutralize one gram of the protein. It can be observed from the table that there are no marked differences in the amount of NaOH required for neutralization of gliadin "A" and gliadin "A" which had been treated with neutral salt solutions. Gliadin "A" requires a *slightly* greater amount of NaOH (14.28×10^{-5} gram equivalents per gram of protein) than does gliadin which had been treated with a neutral salt solution ($11.42 - 12.85 \times 10^{-5}$ gram equivalents), but this difference does not greatly exceed experimental errors. The high values shown by gliadins "B" and "C" (37.14 and 38.56×10^{-5} gram equivalents) are due to the presence of acetic acid adsorbed or combined with the protein and not removable by purification through absolute-alcohol-ether treatments. No increase occurs in the carboxyl groups of the proteins on treatment with neutral salt solutions. Therefore no true hydrolysis of the protein occurs during the peptization process.

The free amino nitrogen in proteins has been studied quantitatively by Van Slyke and Birchard (1914). They concluded that the ϵ-amino group of lysine existed free in the protein molecule and that it could be quantitatively determined by the Van Slyke method for the estimation of amino nitrogen. Nearly all of the α-amino groups which contribute to the amino nitrogen obtained after hydrolysis are bound as peptide linkages in the native protein. Hoffman and Gortner (1925) found gliadin to possess a free amino nitrogen content of 1.86% of the total nitrogen, while one-half of the lysine nitrogen based on the Van Slyke analysis would yield only 0.29% free amino nitrogen.

In this investigation, the free amino nitrogen of gliadin was determined in order to ascertain whether or not any hydrolysis had occurred on peptizing the gliadin with neutral salt solutions. From the data given in Table I, it is seen that the free amino nitrogen of gliadin is slightly higher (2.08%–2.18%) than the value obtained by Hoffman and Gortner (1925). On the other hand, the present series of values are practically identical, that is, the free amino nitrogen of gliadin which had been prepared by either the alcohol or the acetic acid methods, respectively, are nearly the same as the free amino nitrogen of gliadin which had been treated with the various salt solutions.

The ammonia nitrogen of a protein analysis is derived from the

amide nitrogen of the mono-amino dicarboxylic acids and from the deaminization of some of the α-amino groups. Gortner and Holm (1917) have shown that the ammonia derived from the deaminization of the amino groups varies with the length of hydrolysis and that on prolonged hydrolysis, appreciable deaminization of the amino groups may occur giving rise to too high a value for the true amide nitrogen. They have further shown that the amide group of the protein is hydrolyzed in a very short time without appreciable deaminization of the amino groups. In Table I are recorded the "true amide nitrogen" values obtained from gliadin and the various gliadin fractions following a four-hour hydrolysis with 20% HCl. The "true amide nitrogen" values of the fractions are all practically within experimental error of each other. Neutral salt solutions do not produce any change in the amide nitrogen of the gliadin. The solubility differences of gliadin in neutral salts did not produce changes in the ammonia fraction of the protein. Gliadin has been shown by various investigators to possess an ammonia fraction of 25% ± .5%, and the values in Table I agree with those in the literature.

In Table I are recorded the Hausmann numbers of the gliadin fractions, the gliadin samples being hydrolyzed with 20% HCl for 24 hours. The values correspond closely to those of gliadin which other investigators have recorded in the literature. In all of these fractions, the amount of humin nitrogen obtained was exceedingly low, being less than one per cent. The acid hydrolysates of gliadin and the gliadin fractions were light yellow in color, and never a black liquid, which is so characteristic of hydrolysates high in humin. The values of the basic nitrogen are a trifle lower than some of the values recorded in the literature, but are consistent for this series of protein preparations.

The Peptization of Gliadin by Salt Solutions. The data in Tables II and III show that equiionic solutions of the potassium halide salts peptize different amounts of gliadin and confirm the lyotropic series of Cl < Br < I which has been observed in previous papers in this series. The present series of studies, however, point to the conclusion that gliadin cannot, through differential solubility in salt solutions, be fractionated into dissimilar chemical fragments, and the work, taken as a whole, points strongly to the conclusion that gliadin is probably, from the standpoint of organic chemistry, a homogeneous protein, but that the granules of the dry protein or the larger aggregates of the hydrated protein are not physically homogeneous.

As noted in Table II, each extraction with the respective salt solution peptizes less protein than the preceding one, and in every case the fourth extraction hardly peptizes an appreciable quantity of the protein. It is difficult to explain why this should be, for the protein appears to attain a certain hydrated state, but solation decreases on further extraction

with the salt solution. Similar phenomena may frequently occur when biological materials are extracted with salt solutions, and accordingly repeated extraction of a material with a certain salt solution until no more protein is extracted, does not necessarily indicate that the original material is free of that particular protein. As can readily be observed from the data which have been presented, the physical state of the protein has a profound effect on its subsequent behavior toward salt solutions.

Again, it was found in our experiments that the solubility of the protein in a definite volume of salt solution was closely related to the initial amount of protein which was present. We have recognized this and in all cases have maintained the ratio of protein to salt solution at one part to one hundred in order to secure comparable results.

Another point worthy of emphasis is the fact that there exists no sharp line of demarcation between the " soluble " and " insoluble " fractions of the protein as are known to exist in all crystalloids. The values in Table II show that each extraction peptizes less protein, but that a very small amount of protein will continue to go into solution with further treatment with salt solution. The interfacial forces which cause the gliadin micellae to adhere to each other are not all of the same intensity. It would appear that there are at least three series of interfacial forces present in a protein-salt-water system, (1) water-water molecule adhesion, (2) water molecule-protein-micella adhesion, and (3) protein-micella-protein-micella adhesion. The balance of at least these three forces [2] will determine whether a protein gel or a protein sol will result from a given system. The salt-ions undoubtedly have a marked influence on these equilibria which in turn determine whether protein micella adheres to protein micella to form a large aggregate, a gel, or whether the gel aggregate attracts water molecules (or salt-ions) and these in turn bring about the dispersion of the gel to a protein sol (increased " solubility ").

In 1928 Gortner, Hoffman, and Sinclair emphasized the fact that protein " solubility " as defined in the various schemes for protein classification was, at least in notable instances, synonymous with protein " peptization " as that term is used in colloid chemistry and that the nature and concentration of the ions present in solution determine the degree to which a given protein system could be peptized.

Sorensen (1930) has put forward a theory of proteins as " reversibly dissociable component systems." Sorensen's data are essentially in agreement with that of Gortner, Hoffman, and Sinclair (1928), of Staker and Gortner (1931), and with that presented in the present paper. His theory and terminology, however, differ from ours. He disregards the view that the surface forces of colloid chemistry are

[2] We probably have to add water-salt-ions and protein-salt-ions as well.

active in protein-aqueous solution systems and prefers to examine the "solubilities" of proteins on the basis of a hypothesis that "soluble proteins consist of a series of complexes or components, reversibly combined, which makes their constitution expressible by the ordinary formula $A_xB_yC_z\cdots$. *A, B, C,* and so on each marking complete complexes, mainly polypeptides, yet in some cases also containing other groups, for example phosphorus ones, whereas the affixed indices *x, y, z,* and so on, mark the amount to which the indicated complex is present in the entire component system. Within each complex all the atoms and atom groups are linked together by main-valencies, whereas the various complexes in the whole component system are comparatively loosely and reversibly knit together by means of the residual valencies which each component must be assumed to possess, and the strength and nature of which must depend on the chemical composition of the component in question as well as on its physical properties, above all on its dimensions and the resulting shape and surface. But all things considered, the linkage between the components must be supposed to be comparatively slight and of such a nature that alterations in the composition of the solution (salt content, hydrogen-ion activity, alcohol content, temperature) may give rise to reversible dissociations of the involved component systems and interchange of components between the same. When these alterations in the composition of the solution are so suited as to render possible in sufficient quantities the formation of a component system insoluble or sparingly soluble under the new conditions, such a system will be formed and precipitated. In good accord with this is the fact that through suitable proceedings it has been possible to effect a reversible fractionation in the case of all hitherto investigated proteins. In the main the fractions gained possess indeed the properties of the initial material, yet both the physical properties and the chemical composition are more or less modified from fraction to fraction because of their varying contents of the different components."

In most of the experimental work which has been done to date, the evidence for the (organic) chemical heterogeneity of proteins, which have been long regarded as individuals, is very meager. Most of the evidence which Haugaard and Johnson (1930) put forward in an attempt to demonstrate the successful fractionations of gliadins into different *chemical* fractions appears to us not to support their conclusions. Certainly the data in the chemical analyses of their fractions which is reported in their Table 47 (p. 101) show values all of which we regard as being within experimental error of each other with the possible exception of the "acid amide nitrogen" for Fraction I (25.13%) which is approximately 1% lower than for the other three fractions (26.03–26.27).

In regard to such physical properties as viscosity, etc., it is well known that they are greatly influenced by micellar size, previous mechanical and thermal treatment, etc., and that such lyophilic colloids as cellulose nitrate and cellulose acetate may show identical chemical composition and exhibit widely different physical properties in the various fractions. Why should one require that the physical properties of protein systems be explained on the basis of *chemical* heterogeneity in the face of demonstrated *physical* heterogeneity and *chemical identity* in other colloid systems?

It appears probable that Sorensen's "reversible-dissociable component systems" of proteins; Svedberg's "decomposition of protein molecules"; and our "protein peptization" are identical phenomena described in different words by the three groups of workers. Svedberg and co-workers are able to "decompose" protein "molecules" by acids, bases and salt solutions into sols which show, in the ultra-centrifuge, heterogeneity of particle size, these sols of "decomposed molecules" when reworked by a specific technic may later be obtained in the form of sols which contain micelles of only a single particle weight. Haugaard and Johnson report that "it is possible to fractionate a gliadin solution into several other fractions which on recombination give a product with the properties of the original fraction." We have demonstrated that it is possible to peptize gliadin with salt solutions and secure a "soluble" fraction and an essentially "non-peptizable" residue. *If now the soluble fraction and the non-peptizable residue are (separately) reworked and repurified in as nearly as possible the same manner as was the original gliadin preparation, the resulting products are, in a majority of instances, to all intents and purposes, identical in physical properties with the original gliadin preparation.* We regard the differences in peptizability of our gliadin preparations as recorded in Table II as being due to the difficulty of controlling with sufficient exactness the processes of purification, precipitation, drying, etc., so as to produce identically the same state of micellar aggregation in each preparation of the final dry white protein gel. The failure to solve such a problem is not peculiar to the protein chemist. For example, the investigators interested in cellulose esters have faced this same problem in industry and it still remains unsolved.

In the experiments reported in this paper the properties of the original preparation were essentially regained not by a *recombination* of polypeptide fractions as Sorensen's theory requires, but by simply reworking either the "soluble" fraction or the "insoluble" fraction by as nearly as possible the same standard technic which was used to prepare the original sample of gliadin. In this manner the dry purified protein was brought back to approximately the same *physical* state and

the subsequent peptization behavior of the colloid aggregates in the dry gel reflected this similarity in physical state.

Summary and Conclusions

Earlier studies of Gortner, Hoffman, and Sinclair (1928) (1928a) (1929) have already demonstrated that the protein complex of wheat flour could be peptized to different degrees when treated with inorganic salt solutions. From the outgrowth of this investigation, it was decided to study the physico-chemical behavior of a "pure" protein when treated with neutral salt solutions. Gliadin was the material chosen for such a study. This material was prepared by the two established methods, (1) the original alcohol method (first used by Osborne), and (2) the acetic acid method (first used by Blish and Sandstedt).

Pure gliadin was peptized with neutral salts. It has been shown that various concentrations of a particular neutral salt peptized different quantities of protein, and that molar solutions of various neutral salts peptized various amounts of gliadin, KI solutions peptized greater quantities of protein than did KBr solutions, and these in turn possessed a greater peptizing capacity than did KCl solutions.

When gliadin was successively treated with molar KI solution, there remained some protein that was not capable of being peptized with molar KI solution. However, if this non-peptizable residue was purified by repeated precipitations from alcohol in the same manner as in the original preparation of gliadin, the protein was then capable of being further peptized by molar KI solution. Similarly the "KI soluble" fraction when reworked yielded a "KI insoluble" residue.

A study was made of the relative nitrogen distribution of the salt-peptizable and the salt-nonpeptizable fractions, and it was found that the nitrogen distribution was not altered when gliadin was treated with neutral salt solutions, for the Hausmann values were practically the same as those recorded in the literature for untreated gliadin.

The experimental data presented in this investigation appear to substantiate the following statements:

1. The peptization of gliadin by neutral salt solutions appears to reflect the physical state of the protein gel, but not the organic chemical constitution, for the chemical composition of the various fractions is the same, within experimental error, as manifested by the nitrogen distribution (Hausmann values) and other analyses.

2. The treatment of gliadin with the neutral salt solutions which we have used does not produce any "true hydrolysis," for no significant increase occurred in the free amino nitrogen, and no change in the free carboxyl groups.

3. The amount of protein (gliadin) which will be peptized by a

particular salt solution depends upon both the concentration of the salt solution and the amount of gliadin which is present.

4. The lyotropic behavior of the ions on a gliadin-salt-solution system is in the order $I > Br > Cl$.

5. When gliadin is treated repeatedly with molar KI or KBr solutions, there results an extremely "soluble" fraction and an essentially "insoluble" fraction. This difference in "solubility" is accounted for as being due to a *physical* heterogeneity of the protein micelles and not to a *chemical* heterogeneity of mixed protein molecules.

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AN IMPROVED METHOD FOR THE ESTIMATION OF FLOUR DIASTATIC VALUE[1]

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The laboratory control of flour diastatic activity is an item that until recently has not usually been included among the responsibilities of the average American mill chemist, although it has for some years been a common practice among many European flour technologists. However, the introduction of "diastatic control" among many of the larger mills in the United States has created a situation in which the estimation of diastatic values in wheat products is likely to become, of necessity, a matter of routine laboratory practice.

The method proposed by Rumsey (1922) has been generally accepted as being based upon sound principles, and in its original or in modified form has usually been employed in diastatic activity investigations during the past decade. *In principle,* its method is simple enough; it undertakes to measure the maltose produced by one hour's diastasis in a flour-in-water suspension held at a specified and controlled temperature.

Although simple in principle, critical studies of the Rumsey method and its various implications, recently pursued in the writers' laboratory, and involving collaborative testing among different laboratories, have clearly indicated certain disadvantages that need to be overcome in order to put the method on a basis of standardization, convenience, and reliability comparable to other routine tests ordinarily employed by cereal technologists. Although minor difficulties have been encountered with the "blank" determination, the chief source of uncertainty and complications has been the estimation of the maltose produced by diastasis.

Blish, Sandstedt, and Platenius (1929), and Blish (1932) reported a picric acid colorimetric technique as preferable to any of the various copper reduction methods from the standpoint of convenience without loss of accuracy. During the past year, the senior author, while serving as Associate Referee on Diastatic Value of Flour for the Association of Official Agricultural Chemists, submitted a specified colorimetric

[1] Published with the approval of the Director as Paper No. 135, Journal Series, Nebraska Agricultural Experiment Station.

procedure, which had given satisfactory results in this laboratory, to various other laboratories for collaborative testing. The results were so discordant (Blish, 1932) as to eliminate the colorimetric method from consideration as a possible basis for any " official " or " standard " method. A more surprising feature of these collaborative tests, however, was the fact that no better concordance of results was had when the different collaborators employed certain approved copper reduction methods (Blish, 1932).

These experiences have led to the conclusion that neither the colorimetric nor the copper reduction method is likely to serve satisfactorily as a standard " official " testing procedure. The writers have therefore sought a method for estimating maltose values that will, to the highest possible degree, combine the following features: (1) Reliability, (2) simplicity, (3) convenience, and (4) minimum requirement for special equipment. The result of this undertaking has been the establishment of a method which, tentatively at least, appears to meet existing requirements to a degree that is unapproached by any other known method. It is an adaptation of Hagedorn and Jensen's ferricyanide micro-method for the estimation of blood sugar (1923).

Hagedorn and Jensen's (1923) method is based upon the reduction of ferricyanide to ferrocyanide by reducing sugars in alkaline solution. After reduction, the solution is acidified with acetic acid, the ferrocyanide is precipitated as the potassium-zinc salt, and the residual ferricyanide is readily and accurately estimated by adding potassium iodide (which reduces the ferricyanide with the quantitative liberation of iodine) and soluble starch, and titrating with sodium thiosulfate. Standard solutions of ferricyanide and sodium thiosulfate, respectively, may ordinarily be reliably and satisfactorily prepared merely by weighing out and dissolving definite quantities of the pure salts, which are readily obtainable commercially.

Hagedorn and Jensen's method has recently been extensively and successfully applied by a number of workers in the estimation of sugars in different types of biological material. Although its most frequent application has been in studies on animal tissues and products, i.e., blood, urine, etc., several workers, including Hanes (1929), Widdownson (1931), and Lehmann (1931), have found that its principles are equally well suited to investigations with plants and plant products. Minor modifications and adaptations have usually been made to suit special purposes and requirements.

Experimental

Preliminary attempts to apply the principles of Hagedorn and Jensen's (1923) method to the estimation of diastatic activity (maltose

value) of flour, and involving collaborative trials among several different laboratories, gave every indication that the method offers decided advantages over other methods commonly in use. A tentative and hastily-prepared method [2] was devised, and copies of its specifications were sent, together with two samples of flour, to each of a number of laboratories for collaborative trials. The results of these first collaborative tests are shown in Table I.

TABLE I

FIRST COLLABORATIVE TRIAL WITH TENTATIVE METHOD

Collaborator	Diastatic activity	
	Flour A	Flour B
	Mgs. of maltose per 10 gms. flour in 1 hour	
1	328	186
2	328	175
3	303	148
4	332	164
5	309	173
6	327	180
7	310	168

When it is considered that the collaborators were here using (to them at any rate) a new biochemical procedure for the first time, the results in Table I may be regarded as highly encouraging. They are far superior to any results heretofore obtained by the writers with collaborative tests involving other types of diastatic activity methods. The collaborators' special comments also were most favorable. It was therefore decided that an exhaustive study of all details of the method should be undertaken in an effort to arrive at a set of specifications which will combine all desirable features to the highest possible degree.

The further and more detailed studies have resulted in the establishment of an improved and revised set of specifications, which are believed to combine accuracy and reliability with a maximum of convenience and simplicity, both in operation and in equipment needed. A statement of the *preliminary* method is considered unnecessary. Complete specifications for the improved and revised method are herewith presented.

Specifications for the Adopted Procedure

REAGENTS

BUFFER SOLUTION. Three cc. of glacial acetic acid and 4.1 gms. of *anhydrous* sodium acetate are made to one liter with water. The pH of this solution is 4.6–4.8.

[2] Unpublished.

SULFURIC ACID. Ten cc. of concentrated H_2SO_4 diluted to 100 cc. with water (10% by volume).

SODIUM TUNGSTATE SOLUTION. 12% solution of $Na_2WO_4.2H_2O$.

ALKALINE FERRICYANIDE SOLUTION.[3] 16.5 gms. of pure dry $K_3Fe(CN)_6$ and 22 gms. of *anhydrous* Na_2CO_3 in one liter of water. The $K_3Fe(CN)_6$ normality is N/20. This solution maintains its strength for a long period of time if kept in a dark glass bottle away from the light.

N/20 SODIUM THIOSULFATE SOLUTION.[4] 12.41 gms. of $Na_2S_2O_3.5H_2O$ per liter. Select only the clear crystals from the best C.P. grade. If re-distilled *CO_2-free* water (the second distillation being made after the addition of a small quantity of alkaline potassium permanganate to the water, to destroy all traces of organic matter) is used in making up this solution, it will retain its normality for a long time, whereas with ordinary distilled water it is likely to slowly deteriorate on standing.

ACETIC ACID REAGENT. Solution to contain 200 cc. of glacial acetic acid, 70 gms. of KCl, and 20 gms. $ZnSO_4.7H_2O$ per liter.

POTASSIUM IODIDE SOLUTION. To a 50% solution of KI, add one drop of concentrated NaOH for each 100 cc. of solution to prevent or substantially delay deterioration of the solution (with liberation of iodine) on standing, which will otherwise occur. The solution is not fit for use unless colorless.

SOLUBLE STARCH SOLUTION. 1% of soluble starch in 30% NaCl solution. Prepare the soluble starch suspension and pour slowly into boiling water. Add salt and make to volume. The solution should be transparent and colorless.

Procedure

TOTAL MALTOSE AFTER DIASTASIS FOR 1 HOUR

Introduce 5 gms. of flour and a teaspoonful of ignited quartz sand into a 100 or 125 cc. Erlenmeyer flask, and mix flour with sand by rotating the flask. Add 46 cc. of buffer solution, and again mix by rotating the flask until all the flour is thoroughly in suspension. The flour-sand mixture and the buffer solution should be *separately* and *individually* brought to 30° C. before the two are mixed. Digest for one hour at 30° C. preferably in an accurately controlled water thermo-

[3] The best C.P. grade of this salt as purchased on the market may ordinarily be depended upon to be free from moisture and impurities to the extent that it is necessary merely to weigh out the exact quantity required for any specified normality.

[4] Here, as in the case of the ferricyanide, it is ordinarily necessary merely to weigh accurately the quantity specified, owing to the high degree of purity in which the crystalline $Na_2S_2O_3.5H_2O$ may be obtained. Check the ferricyanide against the thiosulfate solution as follows: To 10 cc. of the alkaline ferricyanide solution add 25 cc. of the "acetic acid reagent," followed by 1 cc. of 50% KI, and 2 cc. of soluble starch solution. Titrate with the $Na_2S_2O_3.5H_2O$ solution. It should require exactly 10 cc. of the $Na_2S_2O_3$ to completely discharge the blue starch-iodine color. The $Na_2S_2O_3.5H_2O$ solution may be standardized against pure iodine solution if deemed necessary.

stat, shaking flask (by rotation) every 15 minutes. At the end of the hour add 2 cc. of the 10% (by volume) sulfuric acid solution, and mix thoroughly. Then add 2 cc. of the 12% sodium tungstate solution, mix, and let stand a minute or two. Filter through paper (No. 4 Whatman or its equivalent) discarding the first 8 or 10 drops, and pipette 5 cc. of the filtered extract into a test tube of approximately 50 cc. capacity (18–20 mm. diameter). Add exactly 10 cc. (with pipette) of the alkaline N/20 ferricyanide solution to the 5 cc. of extract in the test tube, and immerse the test tube in a vigorously boiling water bath [5] (the surface of the liquid in the test tube should be 3 or 4 centimeters below the surface of the boiling water). Allow the test tube to remain in the boiling water bath for *exactly* 20 minutes. Then cool the test tube and its contents under running water, and pour at once into a 100 or 125 cc. Erlenmeyer flask. Rinse out the test tube with 25 cc. of the "acetic acid reagent," and add to the contents of the Erlenmeyer flask, with thorough mixing. Then add 1 cc. of the 50% KI solution, followed by 2 cc. of the soluble starch solution, and mix thoroughly. Titrate with N/20 sodium thiosulfate to the complete disappearance of the blue color. A 10 cc. burette is recommended for dispensing the N/20 sodium thiosulfate in this titration. Subtract the number of cc. of N/20 sodium thiosulfate used in the titration from 10, which gives cubic centimeters of N/20 ferricyanide reduced to ferrocyanide by the reducing sugars in the flour extract. This value represents a definite quantity of maltose which may be ascertained by consulting the Maltose Conversion Table. The table has been prepared by applying the specified procedure to standard solutions of pure maltose, using all reagents in the quantities and volumes precisely as employed for flour extracts. The maltose values are given in milligrams. When 5 cc. of flour extract are used, as herein specified, it is necessary merely to multiply the milligrams of maltose by 20 to give milligrams of maltose per 10 gms. of flour in one hour's diastasis. This is the value that is recorded and reported as the measure of the diastatic value of the flour in question.

The foregoing specifications may be used with all ordinary flours whose values for milligrams of maltose produced by 10 gms. of flour in one hour will seldom, if ever, exceed 350. For material giving higher values, such as products from malted or sprouted grain, it is only necessary to use smaller portions of extract, i.e., 1, 2, or 3 cc. instead of 5 cc. In such cases, however, one should add enough distilled water to make up the difference, and one must also use a different factor for converting results into milligrams of maltose per 10 gms. of flour.

[5] The delay between the filtering of the extract and the treatment in the boiling water bath should not be over 15 to 20 minutes at the most. Further delay may cause a slight error due to sucrose hydrolysis in the acid solution.

Thus, if 2 cc. of extract were used, the value obtained from the table is multiplied by 50 instead of 20. In cases where the material in the test tubes is colorless, instead of yellow, after treatment in the boiling water bath, and gives no blue color upon the addition of KI and starch, it is apparent that there was more than enough maltose to reduce all of the ferricyanide, and that the determination must be repeated, using a smaller quantity of extract.

The "Blank" Determination

A "blank" determination, designed to indicate the quantity of reducing sugar originally present in the flour, the value for which presumably should be deducted from the total maltose value after one hour's diastasis, has been generally regarded as an essential step in the estimation of flour diastatic activity. This operation, however, is ordinarily unnecessary when dealing with flour milled from *sound* wheat, because the quantity of reducing sugars originally present as such is so small, and so nearly constant, that it may be disregarded for all practical purposes. The "blank" determination may, therefore, be conveniently omitted in ordinary routine testing. It need be used only when there is occasion to doubt the soundness of the wheat, or in cases where there is known to have been an appreciable quantity of frosted, sprouted, heat-damaged, or otherwise unsound kernels in the wheat from which the flour was milled.

When, however, it is considered necessary to make the "blank" determination, the recommended procedure is as follows: Add to 5 gms. of flour and a teaspoonful of quartz sand, in a 100 or 125 cc. Erlenmeyer flask, 48 cc. of 0.4% (by volume) H_2SO_4;[6] shake thoroughly, and at once add 2 cc. of 12% sodium tungstate. Shake thoroughly again, and after standing two minutes filter through a No. 4 Whatman (or its equivalent) paper. Using 5 cc. of the clear filtrate, proceed according to the specifications for the diastatic activity, hereinbefore described.

Discussion of the Method

It is at once apparent that the method is strictly arbitrary and empirical, and that any deviation from established specifications is almost certain to cause error. This was amply confirmed during the course of many experiments designed to study the effects of varying some of the numerous factors that are operative in the method. These factors were studied individually, and in relation to each other. No detailed account of these experiments or their results will be attempted

[6] It is recommended that the 0.4% H_2SO_4 be pre-cooled to ice-water temperature before adding it to the flour in order to obtain most nearly absolute values. The error is very slight, however, if the pre-cooling is omitted.

in this report, other than to indicate some of the more important factors that have been considered, giving reasons for certain preferences shown in the final selection of the recommended specifications.

BUFFER SOLUTION. For the control of H-ion concentration during diastasis (pH 4.6–4.8) any one of several buffer solutions may be used. The acetic acid-sodium acetate mixture was selected chiefly because in addition to giving satisfactory H-ion control it is the easiest to prepare from simply and readily obtainable ingredients whose composition, purity, and stability may be relied upon. It seems to keep well without the necessity of using toluol or some other preservative.

THE FERRICYANIDE SOLUTION. Ferricyanide solutions of various degrees of normality and alkalinity have been used for the estimation of reducing sugars in biological material. The strengths selected appear to be well suited to purposes of convenience in manipulation.

CLARIFYING AGENTS. The ideal clarifying agent for this purpose should (1) clarify the flour extract perfectly, (2) completely stop all diastatic activity, (3) interfere neither with the subsequent ferricyanide reduction nor the titration, and (4) cause no hydrolysis of sucrose or other soluble substances capable of yielding reducing sugars on hydrolysis.

A number of clarifying agents were studied. These included zinc sulfate in alkaline solution, iron, aluminum, cadmium salts, colloidal iron, trichloroacetic acid, and sodium tungstate in acid solution. Each of these reagents was studied under a variety of conditions as to concentrations of ingredients, concentration of H-ions, etc. Hagedorn and Jensen (1923) used zinc sulfate and alkali (NaOH) for blood work. With flour extracts, zinc sulfate and alkali (preferably Na_2CO_3) can be used, but unless the quantities of $ZnSO_4$ and Na_2CO_3 (also the ratio of one to the other) are carefully adjusted, the filtered extracts are cloudy. When the amounts are properly adjusted, clear filtrates are obtained, which do not undergo change,—due either to sucrose inversion or diastasis,—on long standing.

Some of the other reagents, especially colloidal iron and trichloroacetic acid gave perfectly clear filtrates, and appeared suitable for diastatic value estimations, but for reasons not clearly understood showed tendencies toward high results when used in the " blank " determination of reducing sugars originally present as such in flours.

Each clarifying agent (or combination of reagents) needed a special Maltose Conversion Table of its own.

The acid-tungstate clarification was finally selected as best suited to all conditions and all requirements likely to be encountered in testing flour or other cereal products, including malted wheat and flour. It precipitates the protein far more completely than the $ZnSO_4$-alkali com-

bination, as was shown by quantitative tests, and is very convenient. Aside from the necessity for using a good grade of sodium tungstate,[7] the only essential precaution has to do with the tendency for the clarified extracts to slowly undergo hydrolysis of their sucrose in the presence of the acid. This gives high maltose values if the clarified extracts (either before or after filtration) are allowed to stand too long before being subjected to the ferricyanide treatment. However, this is not an appreciable factor up to 15 or 20 minutes at ordinary room temperature (and is not serious up to 30 minutes), as was shown by a series of careful tests. The extracts can remain for much longer periods (probably several hours) without change, if kept at ice-water temperature.

Reduction of the Alkaline Ferricyanide. Both time and temperature influence the extent of reduction when other factors are equal. Reduction proceeds rapidly during the first few minutes. At the end of 20 minutes its rate has greatly diminished, although further reduction is still possible. The solution should be cooled at once and treated with the "acetic acid reagent" as specified. The remaining operations can be done at the convenience of the technician. Test tubes of uniform size, submerged to uniform depth (3–4 cm.) below the surface of the boiling water are desirable for best results. It is not believed that slight differences in boiling water temperatures, due to normal variations in barometric pressure, will seriously affect the results, although in localities of very high altitude it may be found necessary to prolong heating slightly beyond the time specified (20 minutes).

The Acetic Acid Reagent. The original Hagedorn-Jensen method (1923) calls for acidification with acetic acid, alone, followed by the addition of a solution containing KI, $ZnSO_4$, and a large quantity of NaCl; the purpose of the $ZnSO_4$ being to precipitate the ferrocyanide as the potassium zinc salt, leaving the residual ferricyanide free to oxidize the KI, liberating iodine for titration with $Na_2S_2O_3$ in the presence of starch. Although this procedure can be successfully applied to flour extracts, an attempt to improve certain features of it seemed worth while.

The possibility of substantially reducing the quantity of reagents was considered. Another feature to be dealt with was a tendency to-

[7] The writers have found "Pfanstiehl" sodium tungstate, from the Special Chemicals Company, Highland Park, Illinois, to be a dependable product. The salt should dissolve readily in cold water, giving a solution that is alkaline to phenolphthalein. Failure to meet these conditions indicates that the product consists chiefly of paratungstates. According to Folin (Laboratory Manual of Biological Chemistry, D. Appleton Co., New York, 1922) such tungstates can be rendered serviceable as follows: Prepare a hot 10% solution and allow it to cool. Titrate 25 cc. of the solution with 10% NaOH, using phenolphthalein. The correct end-point is obtained only when the pink color remains for at least 3 minutes. From this one may calculate how much NaOH is required for 100 gms. of sodium tungstate, in order to convert the paratungstate into the simple tungstate. Add the calculated amount, and heat until solution is obtained.

ward the formation of Prussian blue (due to the interaction between ferro- and ferricyanides in acid solution) during the brief interval between acidification and the addition of the KI–$ZnSO_4$–NaCl solution. In the titration with thiosulfate this blue color persisted after the end-point (disappearance of the blue starch-iodine color) had been reached. Ordinarily it is not difficult to distinguish between the two shades of blue, but in occasional instances the color was deep enough to cause uncertainty in identifying the end-point, and it was considered advisable to entirely eliminate the blue color, if possible.

After many trials with various modifications of procedure, it was found advantageous to dissolve certain quantities of $ZnSO_4$ and KCl, respectively, in 20% acetic acid. The use of this reagent combines acidification with the *immediate* precipitation of potassium zinc ferrocyanide. It affects an economy of material and entirely eliminates the formation of Prussian blue. With this modification the titration end-point is a sharp transition from blue to white.

THE KI SOLUTION. Aqueous solutions of KI decompose on standing, with liberation of free iodine. This, of course, can be corrected for by running controls in which water is substituted for flour extracts, and making suitable corrections in the actual flour determinations. However, this decomposition can be avoided if a drop or two of strong alkali is added to the KI solution when freshly made up. Theoretically, this practice might be objectionable on the grounds that on long standing there might be an appreciable accumulation of *iodate*, which would be as effective in oxidizing thiosulfate as free iodine. In practice, however, no evidence of any appreciable iodate formation has appeared, and it is concluded that the preservation of the KI solution by making it mildly alkaline is a reasonably safe procedure. If a slight trace of free iodine appears in the KI solution as freshly made up, it can be discharged by the addition of a few drops of sodium thiosulfate. The addition of a drop or two of strong NaOH will then prevent further liberation of free iodine.

THE SOLUBLE STARCH SOLUTION. A soluble starch solution in water alone works satisfactorily. The use of 30% NaCl solution, instead of water alone, is suggested to insure excellent keeping qualities over a long period of time. Otherwise one must either make up the solution frquently, or use toluene as a preservative.

The Maltose Conversion Table

This is, of course, based upon determinations conducted with carefully standardized solutions of pure maltose.[8] In the preliminary

[8] C.P. "Pfanstiehl" maltose, purchased from the Special Chemicals Company, Highland Park, Illinois.

method that was sent to collaborators whose results are shown in Table I, the maltose conversion table used was made from values secured with pure maltose in water alone. A little later it was found that the table was not strictly applicable to clarified flour extracts containing an excess of sulfuric acid and sodium tungstate. Maltose in the presence of agents ordinarily used for clarifying gives titration values that differ appreciably from those obtained in the absence of these reagents. A table based upon solutions of maltose in water alone, therefore, will not give absolute values for clarified flour extracts. Such values are nevertheless strictly comparative. To obtain maltose values that give the nearest approach to *absolute* as well as comparative values, it was necessary to establish a table using varying quantities of pure maltose in the presence of the same ingredients as employed in the clarification of the flour extracts themselves.

TABLE II

MALTOSE CONVERSION TABLE

0.05N Ferricyanide reduced	Maltose equivalent	0.05N Ferricyanide reduced	Maltose equivalent	0.05N Ferricyanide reduced	Maltose equivalent	0.05N Ferricyanide reduced	Maltose equivalent
Cc.	*Mgs.*	*Cc.*	*Mgs.*	*Cc.*	*Mgs.*	*Cc.*	*Mgs.*
0.1	0.2	2.6	4.2	5.1	8.3	7.6	12.3
0.2	0.3	2.7	4.4	5.2	8.4	7.7	12.5
0.3	0.5	2.8	4.5	5.3	8.6	7.8	12.7
0.4	0.6	2.9	4.7	5.4	8.7	7.9	12.9
0.5	0.8	3.0	4.9	5.5	8.9	8.0	13.0
0.6	1.0	3.1	5.0	5.6	9.1	8.1	13.2
0.7	1.1	3.2	5.2	5.7	9.2	8.2	13.4
0.8	1.3	3.3	5.3	5.8	9.4	8.3	13.5
0.9	1.5	3.4	5.5	5.9	9.6	8.4	13.7
1.0	1.6	3.5	5.7	6.0	9.7	8.5	13.9
1.1	1.8	3.6	5.8	6.1	9.9	8.6	14.0
1.2	1.9	3.7	6.0	6.2	10.0	8.7	14.2
1.3	2.1	3.8	6.2	6.3	10.2	8.8	14.4
1.4	2.3	3.9	6.3	6.4	10.4	8.9	14.6
1.5	2.4	4.0	6.5	6.5	10.5	9.0	14.8
1.6	2.6	4.1	6.6	6.6	10.7	9.1	15.0
1.7	2.8	4.2	6.8	6.7	10.9	9.2	15.2
1.8	2.9	4.3	7.0	6.8	11.0	9.3	15.4
1.9	3.1	4.4	7.1	6.9	11.2	9.4	15.6
2.0	3.2	4.5	7.3	7.0	11.3	9.5	15.9
2.1	3.4	4.6	7.5	7.1	11.5	9.6	16.1
2.2	3.6	4.7	7.6	7.2	11.7	9.7	16.5
2.3	3.7	4.8	7.8	7.3	11.8	9.8	17.0
2.4	3.9	4.9	7.9	7.4	12.0	9.9	—
2.5	4.1	5.0	8.1	7.5	12.2	10.0	—

The maltose solutions used in preparing Table II were standardized by the official Munson and Walker copper reduction method. It is therefore to be expected that the specified ferricyanide procedure and table will give results as close to the absolute maltose values as can

be obtained by the use of the official Munson and Walker method. When milligrams of maltose are plotted against cubic centimeters of ferricyanide reduced, the curve is a straight line up to the point where practically all of the ferricyanide has been reduced, as may be confirmed by consulting the table. Since it is far more convenient to use the table than the chart, the graph is not shown. It may readily be constructed from Table II if desired.

Typical Results with Miscellaneous Flours

In Table III, which follows, are given the results obtained by applying the specified ferricyanide method to 14 miscellaneous commercial flours showing a fairly wide range of diastatic values. Corresponding values obtained by applying the official Munson-Walker method to the same flour series are also shown. The data represent total reducing sugars present in the flour extracts after diastasis for one hour, each value being calculated for 10 gms. of flour, and including the reducing sugar originally present as such in the flour.

TABLE III

MALTOSE VALUES AFTER ONE HOUR'S DIASTASIS, FOR TYPICAL FLOUR SERIES, BY COPPER REDUCTION AND FERRICYANIDE METHODS

Flour number	Maltose values	
	Munson-Walker method	Ferricyanide method
	Mgs. of maltose per 10 gms. of flour after 1 hr. diastasis	
2	324	318
6	164	170
8	216	220
9	181	180
12	160	160
14	170	177
17	127	131
18	208	209
19	215	224
24	224	223
25	227	226
28	172	179
32	215	216
37	225	226

The high degree of concordance between the values obtained by the two methods justifies the conclusion that the ferricyanide method may be depended upon to yield results that are no less accurate and reliable than those secured through the use of the officially approved and accepted Munson-Walker method.

Results obtained by the use of the ferricyanide method have been found to be decidedly consistent and reproduceable, as was confirmed by repeated tests on individual samples.

The "Rumsey Blank" Determination

Blish, Sandstedt, and Astleford (1932) presented evidence showing that the blank determination, designed to measure the amount of reducing sugars originally present as such in flour, is ordinarily an unnecessary step when one is dealing with average flours milled from sound wheat. Kent-Jones (1927) had previously indicated a similar belief. He also suggested the likelihood of sucrose hydrolysis by the acid used to inhibit diastatic activity in conjunction with tungstate. Blish, Sandstedt, and Astleford (1932) confirmed Kent-Jones (1927) in this, and showed the extent of the hydrolysis that occurs during a one-hour extraction period when acid has been added at the start, in order to prevent diastasis. They showed, furthermore, that this hydrolysis may be prevented if the one-hour's digestion is carried out at ice-water temperature, and presented evidence indicating that extraction at ice-water temperature probably gives nearest to the true values for the "Rumsey blank."

In view of the likelihood that occasions for making the "blank" determination may arise—even though it need not necessarily be made a routine procedure—the recent laboratory studies have included an attempt to eliminate the necessity for using the one-hour exraction in an ice-water bath. It was eventually found possible to greatly simplify this step in the manner as indicated in the specifications. By extraction with 0.4% H_2SO_4 at room temperature, and *for only one or two minutes,* one can obtain results closely approximating the values secured by a one-hour extraction in an ice-water bath. This conclusion was based upon data such as are shown in Table IV, in which values resulting

TABLE IV

BLANK DETERMINATIONS BY THE "SHORT" VS. THE "ICE-WATER" METHOD

Flour number	Maltose values	
	Short method	Ice-water method
	Mgs. maltose per 10 gms. of flour	
2	24	21
6	26	22
8	19	16
9	18	15
12	20	18
14	20	14
17	26	21
18	21	20
19	22	18
24	26	22
25	19	18
28	22	18
32	20	20
37	24	21
Malted flour	183	176

from the use of the " ice-water " and the " short " methods, respectively, are compared, using essentially the same flours as were represented in Table III.

It is apparent from Table IV that the " short method " involving only two minutes extraction at room temperature gives values which, although tending to be a trifle higher than those obtained by extraction for one hour at ice-water temperature, are in all probability accurate enough for most purposes. It would seem that complete extraction of the reducing sugars is obtained almost at once, and that there is no necessity for prolonged extraction.

It was subsequently found that if the 0.4% H_2SO_4 alone was cooled to ice-water temperature prior to adding it to the flour in the blank determination, a two-minute extraction gave results that were almost identical with the one-hour extraction in an ice bath. Comparisons of the two procedures with seven different flour samples are shown in Table V.

TABLE V

ONE-HOUR EXTRACTION IN ICE BATH VS. TWO-MINUTE EXTRACTION WITH PRE-COOLED 0.4% H_2SO_4 IN THE BLANK DETERMINATION

Flour number	Maltose values	
	Long method	Short method
	Mgs. maltose per 10 gms. of flour	
8	16	17
9	15	16
14	14	16
17	21	21
19	18	21
24	22	22
Malted flour	176	170

Table V shows that the two methods give almost identical results, the differences being so small as to fall well within the limits of experimental error. Apparently the reducing sugars originally present in flour are almost instantaneously extracted by aqueous solvents.

Ground Whole Wheat

In applying the ferricyanide method to whole wheat, it soon became evident that maltose values on any individual sample of whole wheat are directly proportional to the degree of fineness to which the sample is ground. This of course means that comparative maltose values on whole wheats are significant only when samples are ground to standard or uniform fineness. With samples ground to uniform fineness, it is more than probable that diastatic value estimations on whole wheat

will serve suitably as indices to what may be expected in the flour milled therefrom.

Other Possibilities of the Ferricyanide Method

It should be conveniently possible to adapt the Hagedorn-Jensen (1923) method to advantage in the estimation of Lintner values, diastatic values, and sugars in all types of biological material. It has already been modified to suit a number of purposes, chiefly by European workers.

Summary

1. The ferricyanide method of Hagedorn and Jensen (1923) has been adapted to the estimation of diastatic activity (maltose values) of flour and related cereal products.

2. The method is a superior one from the standpoint of accuracy, reliability, simplicity, and convenience to the technician.

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A FIVE-YEAR SUMMARY OF THE MONTHLY CHECK SAMPLE REPORTS OF THE PIONEER SECTION OF THE A. A. C. C.

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The consensus of opinion among the members of the Pioneer Section of the American Association of Cereal Chemists is that the monthly check sample, in which all laboratories make a quantitative report on certain constituents of one sample, is one of the most valuable activities of the organization; in fact the initial formation of the group centered around these collaborative reports. This cooperative effort has been carried forward continuously, and during the last few years has included moisture, ash, and protein reports from a comparatively large number of cereal laboratories. While a casual examination of these reports may convey valuable information, it is certain that a detailed statistical analysis of them will yield fuller and more convincing conclusions.

Collaborators and Samples

During the first four years of the work included in this study, the only qualification demanded of collaborators was that they be cereal laboratory operatives. They were located principally in the Southwest wheat producing and milling areas, and merely had to request that their name be placed on the check sample mailing list to become a collaborator. Last year, however, each collaborating chemist listed in this report was required to qualify for membership in the American Association of Cereal Chemists.

The reports came from all types of mill, bakery, state, and commercial laboratories, representing improved and unimproved conditions and widely varying personal factors. The group of collaborators can therefore be considered a random one.

The monthly check sample has consisted of a well mixed sample of air-dried flour distributed into the required number of 2 to 5-oz. tin cans, the can being immediately sealed with waterproof tape before mailing to the collaborator. No strict rules have been followed in regard to the specifications of the samples used, the type of flour selected being optional with the collaborator forwarding same. The range of ash has

been from 0.36% to 0.78%, and protein from 7.2% to 15.0%. During the period covered by this report three samples of wheat-meal were sent out, but these are not considered in the present study. No doubt most of the samples were given special mixing before being distributed to the sample cans, but it is possible that some of the samples received no more mixing than is found in a commercial flour, which possibly does not have sufficient mixing of its many widely different streams ahead of the flour packer to give an ideal sample for a collaborative report.

The chemists reporting have taken their turn (alphabetical order) in mailing out the samples and it can be assumed that reasonable care was exercised in submitting the samples.

Group Report Summary

In Table I is given a summary of statistics calculated from the basic data. It will be noted that 2,026 moisture reports, 2,105 ash reports, and 2,558 protein reports are considered covering the five-year period 1928 to 1932, inclusive. When the number of reports involved in this summary is considered, the data given by these figures should form a fairly accurate idea as to what variation might be expected from a random group of collaborators with moisture, protein, and ash determinations, and our present methods of analysis.

In arriving at these figures the elimination of "off" results from the original data was restricted, as nearly as possible, to a rejection of under 1% of the total number of reports. This was exceeded in the case of moisture tests, but all results not used were, in our opinion, so out of line to be reasonably susceptible of suspicion for embodying mistakes. The actual number of analyses not used in the five-year period were 39 moisture reports (1.85% of the total), 22 ash reports (1.02% of the total), and 26 protein reports (0.99% of the total).

From the mean standard deviations in Table I, assuming a normal distribution of variates, we have in the central 0.99% distribution of errors a range of 0.99% for moisture, 0.043% for ash, and 0.49% for protein.

A graphical presentation of the secular change in the standard deviations is given in Figure 1. There is very little indication, indeed, in this graph of the existence of any relationship between the standard deviations for moisture, ash, and protein on the same samples except in the case of sample No. 6, of 1928, in which a high degree of variation in all three tests was found on one sample. This could possibly indicate that the larger sample from which the smaller samples were drawn was not thoroughly mixed, that there was an actual change in moisture content in some of the small samples or that a high systematic and random error existed in all three determinations on this sample.

TABLE I

A Five-Year Summary of the Check Sample Activities of the Pioneer Section, A. A. C. C.

Sample number	Moisture			Ash			Protein		
	Number of reports N	Mean yield $\bar{x}$ %	Stan. dev. σ %	Number of reports N	Mean yield $\bar{x}$ %	Stan. dev. σ %	Number of reports N	Mean yield $\bar{x}$ %	Stan. dev. σ %
1928— 1	27	13.8070	.2466	28	.4546	.0097	33	10.7812	.1147
2	28	13.1017	.1693	29	.3822	.0079	35	11.0137	.0928
3	29	13.9200	.1626	29	.3785	.0082	38	9.6555	.0885
4	27	13.3355	.1927	25	.4613	.0095	32	11.4575	.0563
5	28	11.2210	.1893	30	.3831	.0078	32	9.3679	.1033
6	34	13.0094	.3060	31	.4760	.0162	39	11.0336	.1180
7	26	12.3477	.2422	27	.4893	.0067	35	11.1903	.1034
8	31	13.2590	.1987	33	.4045	.0106	42	10.5416	.1196
9	31	13.0935	.1074	30	.3882	.0074	40	11.6580	.0968
10	30	13.9300	.2082	31	.4185	.0075	35	11.6754	.0828
11	30	11.7276	.1836	28	.4779	.0109	38	13.3508	.0888
12	32	13.1209	.2306	34	.4070	.0068	41	10.9861	.0700
1929— 1	28	13.9900	.1594	30	.3775	.0085	38	11.1371	.0701
2	35	8.8761	.0947	34	.6135	.0076	45	13.8624	.1081
3	34	14.4314	.1332	36	.3963	.0084	45	11.8140	.1051
4	31	13.8766	.1631	34	.4128	.0064	40	11.0400	.0919
5	24	13.2266	.2041	27	.3685	.0098	36	9.9311	.1020
6	28	13.6424	.1618	30	.4294	.0100	39	11.0353	.0998
7	29	13.7580	.2181	33	.3968	.0050	35	11.5454	.1374
8	30	13.6981	.1659	34	.3966	.0080	40	10.6541	.1108
9	34	11.7706	.1714	30	.4131	.0056	42	11.2685	.0929
10	29	12.9114	.1793	32	.4743	.0096	40	11.7244	.0711
11	26	14.7127	.1570	35	.4828	.0074	42	12.1128	.1294
12	32	12.7706	.1430	33	.4110	.0079	41	11.3844	.1110
1930— 1	34	12.5585	.1825	34	.4654	.0078	40	11.1682	.0944
2	35	11.3640	.1760	37	.3950	.0075	46	11.1371	.0713
4	36	12.7864	.1661	36	.4404	.0075	44	11.2631	.0740
5	29	10.1961	.1867	32	.4773	.0168	42	11.0888	.0937
6	29	13.3353	.2294	34	.4007	.0052	41	10.8929	.0875
7	34	13.4664	.1524	34	.4824	.0082	43	10.7430	.1123
8	29	11.9281	.2443	36	.4137	.0064	43	11.4858	.0974
9	37	12.7171	.1959	38	.4478	.0113	44	11.3491	.0641
10	34	13.3543	.1814	37	.4350	.0072	45	11.1255	.0824
11	38	11.6792	.1516	34	.3784	.0092	44	10.7955	.0907
12	34	13.3686	.1131	39	.4427	.0069	50	11.6232	.0879
1931— 2	35	13.2416	.1396	37	.4091	.0061	45	10.9068	.0753
3	40	7.2360	.0908	41	.5000	.0051	48	12.1129	.0725
4	37	13.2666	.2707	38	.3986	.0093	46	11.7041	.0851
5	37	12.0184	.1870	40	.4408	.0121	45	7.2145	.1000
6	38	10.3608	.1573	41	.4668	.0060	48	11.7370	.0925
7	36	12.3381	.1807	39	.4889	.0071	48	13.3785	.0817
8	37	12.6910	.2940	40	.4705	.0068	46	10.9030	.0983
9	48	10.7851	.2204	46	.4012	.0118	50	7.1550	.0850
10	42	13.1985	.2304	44	.4212	.0070	50	11.4188	.0939
11	46	11.9763	.1345	47	.4190	.0081	56	11.1707	.0771
12	49	11.8438	.1488	47	.4284	.0056	57	11.4621	.0752
1932— 1	48	13.3910	.1503	51	.5978	.0098	53	14.9821	.1033
2	44	14.1000	.2342	50	.5690	.0086	62	13.4443	.0852
3	42	13.3400	.1849	44	.4489	.0065	47	10.0218	.0735
4a	37	11.9890	.2043	38	.7833	.0077	45	13.8398	.1185
4b	32	12.2030	.2050	36	.3822	.0098	42	10.6631	.0988
5	41	12.7130	.1495	44	.4600	.0056	52	10.7198	.1118
6	43	13.7520	.2489	43	.4478	.0060	51	12.8611	.1178
8	43	12.4570	.2265	40	.4165	.0095	54	11.3596	.0994
9	43	13.2400	.2901	42	.4134	.0100	51	11.1962	.1160
10	45	12.2650	.1976	44	.4382	.0082	56	12.0280	.0774
11	39	13.6650	.1770	38	.4295	.0104	49	11.6375	.1082
12	42	11.7410	.3933	41	.3898	.0057	52	12.6790	.1238
Total	2,026			2,105			2,558		
	$\bar{\sigma}$		.1911±.0120			.00828±.00052			.0946±.0059

Moisture content is a more or less temporary state and although the tin cans sealed with waterproof tape are very efficient there is no doubt that the adverse conditions which some small samples encountered in transit disturbed the efficiency in retaining a constant moisture content.

When the original data on the above sample were reduced to a common moisture basis the standard deviation remained comparatively high in both the ash and protein determinations. In several other samples of high variation in moisture reports the data were reduced to a common moisture basis and there was very little change in the magnitude and order of variation in ash and protein reports. From this it becomes evident that actual changes in moisture content of the smaller samples are not a strong factor contributing to high variation in ash and protein reports in this type of collaborative effort.

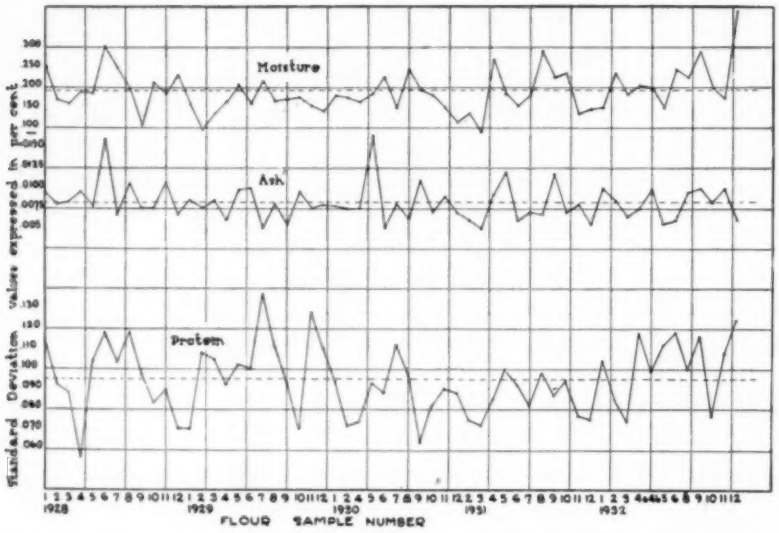

Fig. 1. A graphical analysis of the average variance of group reports on moisture, ash, and protein in the Pioneer Section A. A. C. C. check sample service for the five-year period—1928 to 1932.

Poorly mixed samples would cause a high degree of variation simultaneously in ash and protein, but it will be observed that in very few samples is this the case. This leads to the conclusion that the greater part of the variation between samples is to be accounted for in the combined random and systematic error of analysis.

Treloar [1] describes the random error encountered with a large number of collaborators reporting on one sample. The mean standard deviation of quadruplicate determinations he found to be much lower

[1] Treloar, Alan E. The distribution of errors for the determination of moisture, protein, and ash in flour. Cereal Chem. 9: 449–472.

than the mean standard deviation found in Table I, which includes both the random and systematic error. A careful comparison of these reports gives some idea as to the extent of systematic error in collaborative reports. It must be borne in mind, however, that the actual random error in the present study might possibly be less than the figures presented by Treloar since very few of the reports are based on as many as quadruplicate determinations. This would tend to make the systematic error of even greater magnitude.

It has been a common supposition among the collaborators that a greater variation in the results between laboratories existed during the months of July, August, and September (samples 7, 8, and 9) of each year, when the laboratories were operating, in most cases, in an intense heat. The graphical representation in Figure 1 gives very little evidence of any significant change in the variabilty of results during this season.

Data on Individual Laboratories

Tables II, III, and IV show the standard deviations of the collaborators who reported more or less regularly over a greater portion of the period included in this study. The number of reports by each individual is sufficiently large to give a fairly accurate picture of the personal factor entering into this collaborative work.

The mean standard deviations for this group differ from the mean standard deviations found for the entire group in Table I, but since the differences do not exceed two times their probable error they can be considered of no significance.

In these Tables the ratio of the differences between the individuals' standard deviations and the mean standard deviation of all individuals to the probable error of the mean standard deviation enables us to reject those individuals who contribute more to the mean standard deviation than the average random error. By eliminating all individuals showing a positive ratio of more than three, a second mean is calculated and a second series of ratios permits a further elimination of individuals contributing to the mean standard deviation by more than random error from the remaining collaborators. By again rejecting all positive ratios over three, we have in the moisture determination (Table II) dropped the averages of four individuals from the data and retained 23 individuals with 1,117 reports on 58 samples. From this selected group of collaborators we obtain a third mean standard deviation of 0.1647%, which might well be considered the lowest degree of average variation to be expected with our present methods for moisture determinations. This variation is equivalent to 95% of all variates falling in the range of 0.64% for moisture or 99% of all variates falling in the range of 0.85% for moisture.

In Table III, using the above procedure, we elimnate the reports of 8 collaborators, retaining 19 individuals with 893 reports on 58 samples. The third mean standard deviation of 0.0064% from this selected group can be considered as the lowest degree of average variation to be expected with our present ashing methods. This is equivalent to 95%

TABLE II

AN ANALYSIS OF INDIVIDUAL RECORDS ON 58 PIONEER SECTION COLLABORATIVE SAMPLES FOR MOISTURE DETERMINATION

Coll. number	Number of reports N	Mean error $\frac{E}{\sigma-\bar{\sigma}}$	$\frac{E\sqrt{N}}{.6745\sigma}$	Stan. dev. σ	$\frac{\sigma-\bar{\sigma}}{E\bar{\sigma}}$ 1st series	$\frac{\sigma-\bar{\sigma}}{E\bar{\sigma}}$ 2d series
1	55	+.0101	+ .5	.1888	+ .62	+1.3
2	32	+.1423	+3.7	.3210	+8.73	eliminated
3	58	−.0073	− .4	.1714	− .0	+ .2
4	49	−.0457	−3.5	.1330	−2.80	−2.1
5	58	−.0215	−1.5	.1572	−1.3	− .6
6	53	+.0263	+1.3	.2050	+1.6	+2.3
8	57	−.0421	−3.4	.1366	−2.5	−1.8
9	57	+.0005	+ .0	.1792	− .0	+ .7
10	32	+.0060	+ .3	.1847	+ .4	+1.0
11	56	−.0048	− .3	.1739	− .3	+ .4
12	46	+.0515	+2.2	.2302	+3.1	eliminated
13	53	−.0045	− .3	.1742	− .3	+ .4
14	58	−.0592	−5.5	.1195	−3.6	−2.9
15	27	−.0587	−3.7	.1200	−3.5	−2.9
16	52	+.0159	+ .8	.1946	+ .9	+1.7
17	42	+.0214	+1.0	.2001	+1.3	+2.0
18	51	+.0450	+2.1	.2237	+2.7	+3.4[1]
19	51	+.0092	+ .5	.1879	+ .5	+1.2
20	52	−.0640	−5.9	.1147	−4.1	−3.2
21	33	+.0813	+2.6	.2600	+4.9	eliminated
22	56	−.0309	−2.3	.1478	−1.9	−1.2
23	47	−.0247	−1.6	.1540	−1.5	− .8
24	54	+.0017	+ .1	.1804	+ .1	+ .8
25	36	+.0017	+ .1	.1804	+ .1	+ .8
26	54	−.0339	−2.5	.1448	−2.0	−1.3
27	30	−.0219	−1.1	.1568	−1.3	− .6
31	30	+.0064	+2.8	.1851	+ .4	+1.1
1st total	1,279		First $\bar{\sigma}$	.1787±.0163		$N=27$
2d total	1,168		Second $\bar{\sigma}$	.1672±.0162		$N=24$
3d total	1,117		Third $\bar{\sigma}$	.1647±.0163		$N=23$

[1] Eliminated in calculating 3d mean.

of all variates falling in the range of 0.025% for ash or 99% of all variates falling in the range of 0.033% for ash.

In Table IV, following the same procedure of analysis, 9 collaborators are eliminated and 22 are retained, with 1,087 reports on 58 samples to obtain a third mean standard deviation of 0.076% for protein determinations. From this data we would expect 95% of all variates

to fall in the range of 0.3% for protein or 99% of all variates to fall in the range of 0.4% for protein.

In checking the laboratories that were eliminated in Tables II, III and IV, we find No. 2 rejected on all three counts, Nos. 11, 12, 18, 21, 22, and 31 rejected on two counts, and Nos. 4, 8, 10, 14, 24, and 28 rejected on one count. With 4 rejected in the moisture data, 8 rejected in the ash data, and 9 rejected in the protein data, we have an average probability of 7 rejections in 31 possible instances. Therefore, the average independent probability of rejection on two counts is 1.6 in 31, and we find 6 in 31. For rejection on three counts the theoretical average is 0.4 in 31 and we find 1. Evidence is strong that those too

TABLE III

AN ANALYSIS OF INDIVIDUAL RECORDS ON 58 PIONEER SECTION COLLABORATIVE SAMPLES FOR ASH DETERMINATION

Coll. number	Number of reports N	Mean error E $\sigma-\bar{\sigma}$	$\frac{E\sqrt{N}}{.6745\sigma}$	Stan. dev. σ	$\frac{\sigma-\bar{\sigma}}{E\bar{\sigma}}$ 1st series	2d series
1	55	+.0007	+ .9	.0083	+1.0	+2.3
2	32	+.0064	+3.6	.0140	+9.2	eliminated
3	58	−.0009	−1.5	.0067	−1.3	− .0
4	47	+.0013	+1.4	.0089	+1.8	+3.1[1]
5	58	−.0032	−8.2	.0044	−4.6	−3.3
6	53	−.0001	− .1	.0075	− .1	+1.1
8	57	+.0025	+2.7	.0101	+3.6	eliminated
9	57	−.0027	−6.1	.0049	−3.9	−2.5
10	33	−.0015	−2.0	.0061	−2.2	− .8
11	55	+.0041	+3.8	.0117	+5.9	eliminated
12	47	+.0021	+2.2	.0097	+3.0	eliminated
13	53	−.0022	−4.4	.0054	−3.2	−1.9
14	57	−.0004	− .6	.0072	− .5	+ .7
15	27	+.0002	+ .2	.0078	+ .3	+1.6
16	51	−.0004	− .5	.0072	− .5	− .7
17	43	−.0018	−3.0	.0058	−2.6	−1.3
18	51	+.0001	+ .1	.0077	+ .1	+1.4
19	52	−.0011	−1.8	.0065	−1.6	− .3
20	51	−.0011	−1.8	.0065	−1.6	− .3
21	37	−.0003	− .3	.0073	− .2	+ .8
22	56	+.0026	+2.8	.0102	+3.8	eliminated
23	47	−.0024	−4.6	.0052	−3.6	−2.1
24	54	+.0013	+1.5	.0089	+1.8	+3.1[1]
25	25	−.0017	−2.1	.0059	−2.4	−1.1
26	55	−.0001	− .1	.0075	− .1	+1.1
27	30	−.0027	−4.4	.0049	−3.9	−2.6
31	31	+.0023	+1.9	.0099	+3.3	eliminated

1st total	1,272	First $\bar{\sigma}$	.0076±.00069	$N=27$
2d total	994	Second $\bar{\sigma}$	.0067±.00069	$N=21$
3d total	893	Third $\bar{\sigma}$	.00646±.00076	$N=19$

[1] Eliminated in calculating 3d mean.

variable on one count will also be found too variable on a second and perhaps a third.

An effort was made by questionnaire to determine what effect methods of analysis had on the highly variable results from some laboratories

TABLE IV

AN ANALYSIS OF INDIVIDUAL RECORDS ON 58 PIONEER SECTION COLLABORATIVE SAMPLES FOR PROTEIN DETERMINATION

Coll. number	Number of reports N	Mean error E $\sigma-\bar{\sigma}$	$\frac{E\sqrt{N}}{.6745\sigma}$	Stan. dev. σ	$\frac{\sigma-\bar{\sigma}}{E\bar{\sigma}}$ 1st series	2d series
1	56	−.0309	−5.2	.0660	− 3.7	−1.8
2	33	+.1471	+5.1	.2440	+17.7	eliminated
3	58	−.0426	−8.8	.0543	− 5.1	−3.3
4	48	−.0048	− .5	.0921	− .5	+1.6
5	58	−.0448	−9.7	.0521	− 5.4	−3.6
6	54	+.0036	+ .3	.1005	+ .4	+2.2
7	51	+.0009	+ .1	.0978	+ .0	+2.6
8	57	−.0245	−3.7	.0724	− 2.9	−1.0
9	57	−.0226	−3.4	.0743	− 2.7	− .7
10	33	+.0085	+ .6	.1054	+ 1.0	+3.3[1]
11	54	+.0713	+4.6	.1682	+ 8.6	eliminated
12	47	−.0062	− .6	.0907	− .7	+1.4
13	53	−.0431	−8.6	.0538	− 5.2	−3.4
14	58	+.0388	+3.2	.1357	+ 4.6	eliminated
15	32	−.0471	−7.9	.0498	− 5.7	−3.9
16	52	−.0141	−1.8	.0828	− 1.7	+ .4
17	46	−.0263	−3.7	.0706	− 3.1	−1.2
18	51	+.0283	+2.3	.1252	+ 3.4	eliminated
19	52	−.0205	−2.8	.0764	− 2.4	− .4
20	49	−.0070	− .8	.0899	− .8	+1.3
21	37	+.0375	+2.5	.1344	+ 4.5	eliminated
22	57	+.0433	+3.4	.1402	+ 5.2	eliminated
23	47	+.0021	+ .3	.0990	+ .2	+2.5
24	53	−.0001	− .1	.0970	− .0	+2.2
25	37	−.0159	−1.8	.0810	− 2.0	+ .1
26	55	−.0353	−6.3	.0616	− 4.2	−2.3
27	30	−.0055	− .5	.0914	− .7	+1.4
28	20	+.0075	+ .5	.1044	+ .9	+3.2[1]
29	50	−.0355	−6.0	.0614	− 4.3	−2.6
30	45	−.0286	−4.1	.0683	− 3.4	−1.5
31	30	+.0061	+ .5	.1030	+ .7	+3.0[1]
1st total	1,460		First $\bar{\sigma}$	.0949±.0083		$N=31$
2d total	1,170		Second $\bar{\sigma}$	.0798±.0076		$N=25$
3d total	1,087		Third $\bar{\sigma}$	.0765±.0077		$N=22$

[1] Eliminated in calculating 3d mean.

and the low variation in others. The information received was too inadequate to give any definite light on the subject, however, the following information is of some value as a part of this study.

The number of replications made by all collaborators in reporting their results were, in terms of per cent of the total number reporting:

For moisture, one—48%, two—48%, three—4%; for ash, one—13%, two—80%, three—7%; and for protein, one—2%, two—58%, three—20%, four—16%, five—2%, ten—2%. From these figures we calculate the following average number of replications made in reporting the three constituents: Moisture 1.6, ash 1.9, and protein, 2.7.

The average number of replications made by those collaborators who were rejected on as many as one count, was, for moisture 1.5, ash 2.0, and protein 2.4. It can readily be seen that the random error might be expected to be a greater factor in the variability of reports on moisture than on protein reports since the accuracy of a mean increases in proportion to the square root of the number of replications. In the study of the individual records there was little indication that a small number of replications was responsible for certain collaborators being rejected. The particular individuals who used one and ten determinations respectively, for their reports on protein, showed variability very close to the average. Eighty-five per cent of all the collaborators treated the sample in a routine manner and 15% gave special attention to its analysis.

In the methods for moisture, 65% were using the air-oven method and 35% were using vacuum ovens. A wide range of treatment was reported by those using vacuum ovens.

Eighty-five per cent of the laboratories were weighing ash by the direct method.

Protein methods used were numerous modifications of the Kjeldahl method with a bare majority using the procedure described in the A. A. C. C. Methods for the Analysis of Cereals and Cereal Products.

Conclusion

The collaborators reports of the Pioneer Section for the past five years indicate that an average range of 0.99% for moisture, 0.043% for ash, and 0.49% for protein would include the error distribution of 99% of all the quantitative reports on these constituents.

A change of actual moisture content in small samples and heterogeneity of larger samples appear to be insignificant factors in the reports involved in this study.

When laboratories showing high random error are eliminated, these data indicate that the best that can be expected from such collaborative reports is an average variation in which a range of 0.86% for moisture, 0.033% for ash, and 0.4% for protein would include the error distribution of 99% of all reports. This is equivalent to a 95% central range of error distribution of 0.64% for moisture, 0.025% for ash, and 0.3% for protein.

Laboratories showing high variability on one count are very likely to have high variability on a second count and perhaps a third.

One important factor in the lack of concordant results within a group of laboratories is the high random error encountered in some few laboratories.

When Treloar's (1932)[2] definition of the magnitude of the average random error in a group of collaborators is compared with the total variation in both random and selected groups in this study, it is apparent that high systematic error is a strong contributing factor to the variability in collaborative reports.

[2] See footnote 1, page 206.

HIGHER VERSUS LOWER MATHEMATICS IN INTERPRETING BAKING QUALITY [1]

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I wish to take you into a most disturbing field of human endeavor. It is a field of science where quantitative results are obtained. Mathematics is necessarily used. Yet in this field we find that many of the most elementary principles of mathematics do not apply. For example, $5 + 7$ does not necessarily equal $4 + 8$. Things equal to the same things are not necessarily equal to each other and the whole is seldom equal to the sum of all its parts. I refer to the field of the baking test and the utilization thereof.

My object is to invite attention to some considerations surrounding the interpretation of the baking test. On examination it may be thought that these considerations are perhaps self-evident but experience shows that they are frequently overlooked, often leading to serious error. If some of these ideas are disturbing, that is no reason why we should not face them. We have all been very busy making baking tests. Hundreds, perhaps thousands of baking tests are being made every day and we are accomplishing a great deal with these tests. However, as Freeman (1931) says: "Experimenting does not absolve the scientist from the duty of thinking."

Our present difficulty arises in large part from our early training. At school and college we study mostly very simple conditions. We have difficulty in realizing that these conditions do not always obtain in later life. We are taught certain axioms and come to believe that they are universally true. We are seldom taught that these axioms are true only in some branches of pure mathematics and they are by no means true in many fields of applied science as Cohen (1931) and other have pointed out.

In studying analytical chemistry we start with some very simple determination. My own first quantitative determination, for example, was the percentage of chlorine in NaCl. There is but one answer to this problem. This answer may be represented by a point. All competent chemists will get essentially the same answer to this question

[1] Read at the Annual Convention of the Association of Official Agricultural Chemists, Washington, D. C., November 1, 1932.

and the only possible way to improve our answers is in adding another decimal place of significant figures. Nearly all of the analytical chemistry which we do in college is of this type where there is one answer and the only problem is to get it. We naturally come to feel that all analytical problems must be of this nature and that they must have one single correct answer. After such a training, it is difficult for us to understand the determination of baking quality where there are many answers, all of equal correctness. In fact, we shall find that in studying baking quality very little pure mathematics can be applied without modification.

Addition and the Baking Score

In pure mathematics we can add together any series of integers. For example, if I put down

$$\begin{array}{r} 4 \\ 5 \\ 6 \\ \hline \text{The total is.....} \ 15 \end{array}$$

We sometimes do not realize that by making these integers represent quantities in applied mathematics it may become impossible to add them. For example, what mathematician can give the total of the following:

$$\begin{array}{r} 4 \text{ minutes} \\ 5 \text{ grams} \\ 6 \text{ inches} \\ \hline \text{Total.....} \ ? \end{array}$$

In arriving at the score of a loaf of bread, which score is based on loaf volume, color of the crumb, etc., we come dangerously near adding cubic centimeters to color in order to get dollars as the total in a single figure baking score. It is true that volume can be measured in cubic centimeters, color can be measured, texture can be measured, but it is certainly true that these quantities can *not* be added as they stand. In order to add these, each one must be expressed as a ratio—its relation to perfect—that is, they must be expressed as degree of perfection (Platt, 1931). From this we see at once that some standard of perfection must be understood or our score is meaningless. This brings out the importance of the standard of perfection chosen, as this is a factor in each ratio.

Expressed in another way, in arriving at a single figure baking score, we really add the *desirability* of the loaf as judged by its volume, to the *desirability* as judged by its crumb color, to the *desirability* as judged by other factors, until we arrive at one figure which represents the general *desirability* of the loaf as a whole.

We see that this is a case where $4 + 8$ is not equal to $5 + 7$. For example, a flour giving a small volume but good color may have the same total score as a flour giving a large volume but poor color, but these two flours are not equal in any characteristic nor are the indications for their use in any way whatever the same.

Single figure baking scores, as recommended by Larmour (1929), are very useful and save much time and mental effort, but in cereal chemistry they must always be used with a tremendous mental reservation. Without this, their apparent simplicity is, in fact, misleading.

A Dangerous Axiom

"Things which are equal to the same thing are equal to each other." This was one of the first axioms of Euclid, but Euclid was certainly not a cereal chemist. The attempt to apply this axiom to certain fields of applied cereal chemistry has led to disasrous consequences in many large companies.

Supposing we have three cakes, A, B, and C, and supposing our scientific instruments for measuring differences are those exceedingly delicate instruments, the human senses of taste and smell. Supposing A is our standard cake and we cheapen it by leaving out a little butter. We take out so little butter from our formula that when measured with the instruments described, there is no difference between A and B. It is, of course, well known that out of a large batch you can omit a few pounds of butter, replacing it by some other shortening and you can not demonstrate any significant difference in the cakes as shown by the judgment of any one man or the average scores of a number of competent judges. Judged by our yardstick, therefore, you can actually prove mathematically, using as much statistical analysis as you choose, that A is equal to B.[2] Supposing that you now cheapen the B formula by a similarly imperceptible amount, making a new cake C which has in turn slightly less butter than B, but not enough less to be detected. Here again C is equal in its impression on our senses to B, in that no judges can tell the difference. It is quite probable, however, that using this same method of judgment and these same judges you will find a significant difference between A and C. We have here a very clear and common example of a case where two things (A and C) are equal to the same thing (namely B) but are not equal to each other, using the same standards of measurement in each case.

This error is frequently seen. A formula is cheapened by leaving out a small quantity of some ingredient and the two cakes are compared with one another without noticing any significant difference. This

[2] Put more exactly, the impression which A makes on our senses is equal to the impression which B makes. So also with the equal impressions made by B and C.

second formula is then cheapened, and then recheapened. In each case the product is compared with the product immediately preceding it, and not with the original. This is a point of great practical importance.

I am reminded of a biscuit company, making perhaps five hundred 5-barrel batches of soda crackers daily. At the time in question flour was worth about 2c and lard about 12c a pound. One of the officers of the company figured out how much money could be saved providing that out of each 5-barrel batch they took one pound of lard and added one pound of flour. He demonstrated truthfully that this would save $50.00 a day, that there would be no loss in yield of crackers, and that no one could tell the difference in the crackers. It was at first difficult to see the fallacy in this argument and the question arose if this procedure was desirable, why not continue taking out 2, 3, and 4 pounds of lard, etc. The fallacy consists in applying an axiom of pure mathematics to applied cereal chemistry. It touches on the fundamental principle of integral calculus, namely, "If you take enough nothings, their sum is something."

I would invite your attention also to the fact that only in pure mathematics is the whole equal to the sum of all its parts. In most fields of human endeavor the whole is seldom equal to the sum of all its parts and is usually greater than this sum. For example, the impression made on our consciousness from the sound of a man's voice talking two blocks away is absolutely zero. This sound does not arise above the threshold of our consciousness and so makes absolutely no impression on us whatever. However, the sound of a thousand people talking is by no means $1000 \times 0 = 0$ but has a definite effect on our consciousness.

In the same way consider a man standing in front of a large cathedral on a perfectly dark night, so that he can not see the cathedral. Supposing that he has a small brilliant spotlight, so that he can see any detail of the building. Such a man could certainly examine in turn every window, every door, and every detail without having seen the cathedral or having any clear perception of what it was like. In the same way the taste of a product such as cake is, I think, more than the sum of a little saltiness and a little sweetness and a little wheatiness, etc. A cake has a taste of its own as a whole, not merely as a combination of elementary tastes.

Baking Qualities

We come now to perhaps the most important respect in which interpretation of baking quality varies from the very simple quantitative determinations that we have been accustomed to in most of our other experience in quantitative analyses. In ordinary quantitative deter-

minations there is one correct answer which can be represented by a single point on a line, and our whole object lies in arriving at this one correct answer. In both theoretical and practical work, there are degrees of precision which it is necessary to attain. Once the necessary degree of precision is obtained, nothing whatever is gained by additional determinations. This is not true in baking tests where every additional test made under different conditions adds something to our knowledge.

I used before the example of the determination of chlorine in sodium chloride, a very easy procedure. On the other hand, a determination may be very difficult to make and the results obtained by different competent chemists may be widely scattered, and yet there may be just *one* correct result which we can approach as closely as we wish, depending upon the amount of time and skill which we devote to the problem. In the determination of lactose in milk bread, for example, there is one certain definite amount of lactose in the bread, even though our individual results may be quite variable and far from the correct figure. Most determinations then, whether simple or complex to carry out, have just one answer. Such determinations are sharply differentiated from the determination of baking quality which has many answers and which can never be adequately represented by a single point.

In making a determination of baking quality, it is highly desirable and indeed necessary to start with one standard baking test, giving one result. We can attain any precision that we desire in this one test and we can repeat it as often as we wish and thereby reduce the probable error of the mean to any figure that you care to name, but with all this effort this one test can never tell us the whole story in regard to the baking quality of a flour because these replications of this one test are concerned entirely with the accurate location of one point, whereas the baking quality of a flour can only be properly represented by a large number of points. The necessity for two or more points was pointed out in the beginning of our standard bread baking test where a modification of the basic test was proposed to show the " response to bromate." In a similar way the response to sugar has been pointed out by Blish and Hughes (1932), who state:

> " What Jørgensen and other critics of the standard A. A. C. C. procedure apparently fail to appreciate is that the so-called 'basic procedure method' is merely a reference method, and is, as pointed out by Werner (1925), a 'foundation for subsequent tests.' The method as adopted by the A. A. C. C. distinctly embodies 'Additional and Supplementary' tests. Four such tests are indicated, but it should require no great stretch of the imagination to recognize that other supplementary tests might well have been included. The suggestion of supplementary tests is merely in recognition of the obvious fact that starting from an established foundation it is permissible to vary any one factor at a time without violating any principles of scientific laboratory testing."

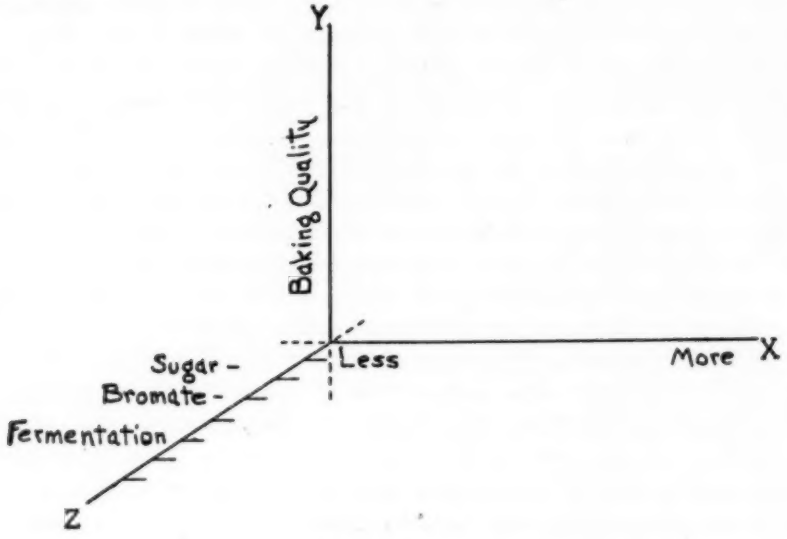
What is true of the bread baking test is doubly true of any cake baking test.

From the point of view of the flour manufacturer, we are forced very reluctantly to agree with Werner (1932) who stated:

> "A flour of say, 13% moisture, 0.42% ash, 11% protein, and other analytical indexes to which you may help yourself, ad lib., when used in different or even the same formula, may have and in fact does have as many baking qualities as there are bakers operating in different climates under different conditions on different days with different formulae, different absorption and different mixing, fermentation or proof time."

This expresses the gist of my present remarks.

The picture of baking qualities that I find most helpful is the following:



I think of the points making up the baking quality of any given flour as lying on an undulating surface in three dimensions. The Y co-ordinate or height of the surface above "sea level" represents the goodness of the baking quality. The infinite number of points in the surface represents the infinite number of formulas and methods by which that baking may be determined. Just as the height of the surface may or may not be the same at different parts, so the baking quality may or may not be the same when tried under different conditions. Blish and Hughes (1932), for example, have pointed out that the baking quality of a flour when tried with a long fermentation without additions of sugar, or diatase may be poor, but with such additions, may be good.

Along the X axis, going from left to right, I imagine the various factors used in increasing amounts, for example, increasing amounts of sugar, of mixing, or of fermentation, etc.

Along the Z axis, I imagine laid off starting points for the various factors we are to try. We try, for example, the effect on baking quality of bromate, sugar, malt extract, milk, of fermentation, mixing, etc., and each of these factors is tried from very little to very much. So we get our " Surface of Baking Qualities " in three dimensions.

Now in studying this surface, it is evident that we may study one point very carefully so as to locate its " altitude " very exactly. This is what we do when we take a single standard baking test and make a careful study of results from this one method. Or you may make a broad study of how a given flour will act under a great variety of conditions, in other words, determine the baking qualities at a great many points on our surface, and so get its tolerance to fermentation and mixing, etc. Both procedures are necessary. The analogy to topographic mapping is evident, where the altitudes of a few points (bench marks) are exactly determined, but the topography of the intervening country is also surveyed.

It is evident that no one point, however chosen or studied, can possibly tell the whole story. The more points we determine, the more we know about the characteristics of the flour in question. It is also evident that certain parts of the surface are of great theoretical and practical interest, while other parts have comparatively little interest.

In considering the relative baking quality of two flours, A and B, we see that the surface of A may be at all points above the surface of B, or the surfaces may intersect, showing that under some conditions A is better and under other conditions B is better. This is a common but somewhat disturbing fact. This fact is implied in a great deal of modern cereal chemical research, especially in the excellent work being done by the Committee on Methods of Testing Cake and Biscuit Flours (for example, L. H. Bailey, 1931). When we see that the relative standing of Sample A and Sample B may be reversed by a change in the method, we realize the tremendous importance of the choice of the methods to be used for baking tests.

Examination shows that the so-called " Surface of Baking Qualities " just described is not continuous along the Z axis where we lay off first a study of response to bromate, then of response to sucrose, etc. It is also difficult, as we know, to represent a three dimensional surface clearly on paper. Therefore, for practical purposes, baking qualities are best laid off on two axes. As before, the ordinates are the baking qualities. The abscissae are the amounts of the various added factors. To represent a study of these separate factors, however, we use different lines, showing the difference by making colored lines, dots, etc.

The depiction of baking quality in three dimensions has been much more thoroughly developed by Landis and Frey (1932).

To make the contrast clearer between a determination in ordinary chemical analysis and the determination of baking quality, let us consider that we wish to find out whether sodium chloride or potassium chloride contains the most chlorine. Any competent chemist will find sodium chloride to contain 60.7% of chlorine and potassium chloride to contain 47.6% of chlorine. Except for gaining a few unimportant decimal places, we know that there would be no advantage whatever in asking a number of chemists to repeat this determination. We feel perfectly sure that no competent chemist would ever find that KCl contained more chlorine than NaCl. On the other hand, if we take two good commercial samples of flour, A and B, and wish to state which has the better bread or cake baking quality, we will probably find as follows:

In some bakeries, we will find no difference.

In some bakeries we will find that A will give better results by their regular method. However, if we modify this method, we will often find that results from B can be much improved. With certain modifications B may even show better than A.

In other bakeries B will give better results by their regular method. Here modifications of formula and method may improve the showing of A.

In short, kinds of cakes, methods of beating, ingredients used, all vary so much that results from baking tests must necessarily show a wide variation. If A is very good and B very poor, the differences will be more clean cut. If the difference between them is less there will be more discrepancies and it will be likely that A will suit some formulas better and B will suit other formulas better.

If differences are small when we say " A has better baking qualities than B," all we mean is that *A will give better results than B in the majority of bakeries.*

The more widespread the conditions under which these baking qualities are tested the more complete is our knowledge about them. The more definitely we know the conditions under which a sample of flour is to be used, the more accurately we can state its baking qualities under those conditions.

We constantly find this condition in laboratory baking and still more in commercial baking. For example, in the collaborative testing of four cake flours reported by Brooke (1929), three of the flours were each put in first place by some collaborator and last place by some other. The excepted flour was placed first, second, and third by different collaborators, though not actually last.

After all, the term baking quality is really the sum of a number of entirely separate characteristics. In the future perhaps we will dwell

more upon these separate characteristics, such as protein, ash, color, diastic power, etc., and less on the total baking quality, the real meaning of which is not so exact.

It is evident that many ingredients other than flour have bread- or cake-baking qualities which we may desire to determine. We find wide differences in the bread-baking quality of yeast, malt, and milk. For cake baking tests are made on eggs, milk, and baking powder. Each ingredient may have many different baking qualities depending partly on the purpose for which it is used.

Conclusions

1. Our early training in mathematics taught us certain simple truths which are seldom found to be unqualifiedly true in applied science. Similarly our early training in chemistry dealt with ideally simple questions. In the baking test, conditions are not simple and many of our early points of view are inadequate.

2. Baking quality should be spelt with a final "s." It is not a point but a large series of points. Similarly, baking quality itself is not one characteristic but is made up of a series of entirely separate characteristics.

The only picture that can approach adequacy and avoid being positively misleading is one showing these different characteristics and showing baking quality under several different conditions. For simplicity and economy of time and mental effort we must often omit the details and deal with a single baking quality expressed as a single figure. Whenever we do this, however, we must make tremendous mental reservations.

3. It is part of our difficult job as cereal chemists not only to recognize these facts ourselves, but also to educate those practical men to whom we report our baking determinations. All this must be done without appearing to insert an "alibi" into every statement that we make.

In closing, I will borrow another phrase from Dr. Werner. To take a single figure for baking quality at its face value and to treat this figure like a definite number subject to the laws of pure mathematics is to invite "premeditated failure".

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THE RELATIONSHIP BETWEEN ASH CONTENT AND THE PEPTIZABILITY OF WHEAT FLOUR PROTEINS OF WESTERN CANADIAN HARD RED SPRING WHEAT

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In recent years several investigators have demonstrated that the proteins of wheat are colloidal in nature and that differences in the strength of wheat flours are very probably due to a great extent to differences in the colloidal state of their proteins.

A classification of proteins, based on solubilities in various media, has been adopted by a joint committee of the American Physiological Society and the American Society of Biological Chemists (1908). According to this classification, those fractions soluble in solutions of neutral salts of strong acids and strong bases are defined as globulins. Most text books make the definition more complete by stipulating that *dilute* solutions be used. No mention, however, is made of what salts are to be used.

The old idea of protein solubility must be questioned when looked at from the colloidal standpoint. Gortner (1927), Hoffman and Gortner (1927), and Gortner, Hoffman, and Sinclair (1928, 1929) have shown that the amount of protein which can be extracted from a flour varies with the salt used and with the concentration used for extraction. Their data clearly demonstrates that protein "solubility" is really not solubility in the strict sense of the term, but is protein peptization, and that the phenomena is of a colloidal nature.

One of the striking features of their data is the fact that the proteins of the flours studied showed a pronounced variability in the ease with which they were peptized. In searching for an explanation, the authors came to the conclusion, that in all probability differences in the ease of peptization was the result of differences in the colloidal natures of the proteins, yet they were unable to offer any suggestions or explanations as to the reasons underlying their occurrence. They suggested that the differences probably had their origin in the environmental factors under which the wheat was grown and harvested.

Geddes and Goulden (1930), using the method as outlined by Gortner, Hoffman, and Sinclair(1929), studied a series of 102 samples of experimentally milled flours selected at random from the 1929 Western Canadian crop. Twenty samples representing each commercial wheat grade, from grade No. 2 Northern to grade No. 6, were utilized. Using N/2 $MgSO_4$ solutions, they found only small differences between the peptizability of the flour proteins, indicating that there was no essential difference between the peptizability of the proteins from sound and immature, or damaged wheat, although baking tests indicated that the quality progressively decreased with decreasing grade of the wheat. Table I summarizes their data.

TABLE I

PERCENTAGE OF PROTEIN PEPTIZED BY N/2 $MgSO_4$ IN 102 SAMPLES OF EXPERIMENTALLY MILLED FLOURS CLASSIFIED ACCORDING TO THEIR COMMERCIAL WHEAT GRADE. (DATA OF GEDDES AND GOULDEN, 1930.)

Wheat grade	No. 2 Northern	No. 3 Northern	No. 4 Northern	No. 5	No. 6	Entire series
$\bar{x}$, protein peptized	2.29	2.24	2.20	2.31	2.25	2.26
σ, protein peptized	0.111	0.155	0.152	0.190	0.150	0.158

The effect of KBr and KI solutions was studied with some of the flours with similar results.

MacLeod (1929) studying a series of pure strain Marquis wheat grown from the same seed but under varying environmental conditions, also found but slight differences in the peptizability of his series of samples.

Harris (1930, a, b, c, and 1932) studied the peptization of wheat flour proteins with several series of flours. His method of study was simpler than that of Gortner, Hoffman, and Sinclair (1929), and was necessitated by the absence of a centrifuge. With a series of 44 experimentally milled flours from Marquis wheat Harris (1930) obtained only a small range in the amount of protein peptized by N/2 $MgSO_4$ and KBr solutions. With another series of pure strain Marquis wheat flours, similar to those of MacLeod's study, he obtained similar results.

However, with a series of commercial flours (Harris, 1930 a) milled mostly from Western Canadian wheat, the amount of protein peptized (as per cent of flour) ranged from 1.78% to 2.58% when $MgSO_4$ was used, and from 3.10% to 4.97% when KBr solutions were used—a significant difference in peptizability. With a series of twenty mill stream flours, greater differences were obtained. The range in peptizability found by Harris is shown in Table II.

TABLE II

RANGE OF AMOUNT OF PROTEIN PEPTIZED FROM VARIOUS FLOURS WITH THREE DIFFERENT SALT SOLUTIONS. (DATA OF HARRIS, 1930, a.)

Series	Range of peptizability in per cent Salt used		
	$MgSO_4$	KBr	KI
31 commercially milled flours	1.78 to 2.58	3.10 to 4.97	——
20 mill stream flours	1.95 to 6.67	2.25 to 8.90	5.80 to 9.67

Pascoe, Gortner, and Sherwood (1930) examined the peptizability of the flour streams of the Minnesota State Flour Mill and obtained a wide range in the amount of protein extracted by four salt solutions. Their data are given in Table III.

TABLE III

RANGE IN THE PROTEIN EXTRACT BY SALT SOLUTIONS FROM THE MILL STREAM FLOURS OF THE MINNESOTA STATE FLOUR MILL. (DATA OF PASCOE, GORTNER, AND SHERWOOD, 1930.)

Range of protein peptized in per cent Salt used			
KF	KCl	KBr	$MgSO_4$
1.81 to 6.45	2.44 to 6.66	3.45 to 7.63	2.27 to 6.30

Jacobs (1915) reported large variations in the percentage of protein "soluble" in 5% K_2SO_4 solution, in the instance of the mill streams of a Minneapolis mill.

In general, the reported literature indicates, that for experimentally milled flours the percentage of protein peptized by neutral salt solutions (computed as per cent of flour) does not differ very greatly from one sample to another, for any given salt. When the series is composed of commercially milled flours or of mill streams flours, the range may be considerably widened.

In an investigation of the hydration capacity of wheat flour proteins, Rich (1932) showed that ash content of flour played a very important part in determining the hydration capacity of the samples of flour

studied in his series. The published literature on the peptization of wheat flour proteins suggested that ash content might also play an important role in determining the amount of protein that be peptized by a neutral salt solution. Accordingly, the available literature was examined in order to determine whether any relationship existed between ash content and peptized protein. Examination of the data of Gortner, Hoffman, and Sinclair (1929) showed that there was a definite trend towards increasing amounts of protein extracted by salt solutions with increasing ash content. An exception was noted in the case of sample No. 17, which was milled from durum wheat, and therefore not a bread wheat flour.

The coefficients of correlation between ash content and the amount of protein peptized by different salt solutions obtained from the data examined are given in Table IV and in Figures 1, 2 and 3.

TABLE IV

CORRELATION BETWEEN ASH CONTENT AND THE AMOUNT OF PROTEIN PEPTIZED BY DIFFERENT SALT SOLUTIONS

Data involved	Peptizing agent	r_{xy} calculated from data	Value of r_{xy} at the 5% point
Gortner, Hoffman, and Sinclair (1929)	KF	+0.821	
	Na_2SO_4	+0.914	
	KCl	+0.710	
	$MgSO_4$	+0.734	+0.602
	KBr	+0.430	
	KI	+0.214	
Harris (1930 a) commercially milled flours	$MgSO_4$	+0.744	
	KBr	+0.585	+0.349
Harris (1930 a) mill stream flours	$MgSO_4$	+0.952	
	KBr	+0.887	+0.448
	KI	+0.392	

The data of Pascoe, Gortner, and Sherwood (1930) does not include the ash content of the mill stream flours used in their experiments, but, judging from the names of the streams, which were given, the relationship was very probably of the same order as that obtained by Harris (1930 a) with mill stream flours.

Examination of the figures and the coefficients of correlation indicates that the correlation between ash content and the amount of protein peptized is the least for those salts having the greatest peptizing effect.

Experimental

In order to ascertain whether the conclusions reached by examination of the published data were general, three sets of mill stream flours were

extracted with N/1 $MgSO_4$ solution, using the procedure outlined by Harris (1930). This method was necessitated by the absence of a mechanical shaker and a centrifuge, and consisted of adding 150 cc. of the $MgSO_4$ solution to 6 gms. of flour in an Erlenmeyer flask. The

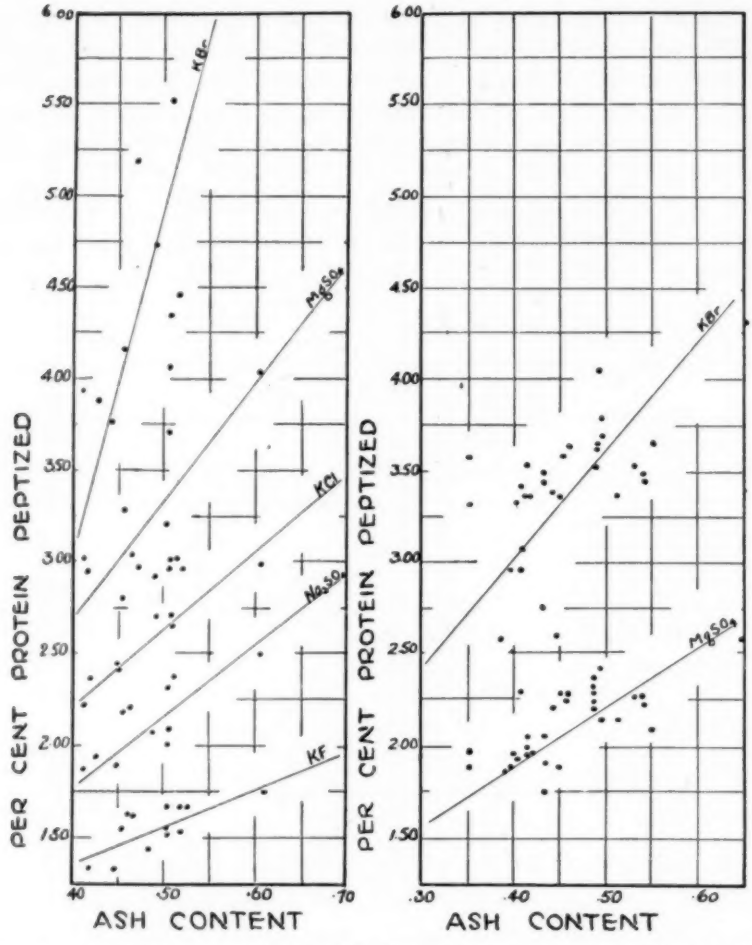

Fig. 1. Ash content of commercially milled flours and amount of protein peptized by different salt solutions (from data of Gortner, Hoffman, and Sinclair, Cereal Chem. 6: 1–17).

Fig. 2. Ash content of commercially milled flours and amount of protein peptized by N/1 $MgSO_4$ and KBr solutions (from data of Harris, Cereal Chem. 8: 113–133).

suspension so formed was shaken at intervals of five minutes for one hour to prevent settling of the suspended particles. The flask was then set aside for several hours to allow the suspended particles to settle. At the end of this period, the clear, supernatant liquid was decanted from the flour, and the protein determined by the Kjeldahl-Gunning

method. The data obtained are given in Table V. The correlation between ash content and the amount of protein peptized is given in Table VI.

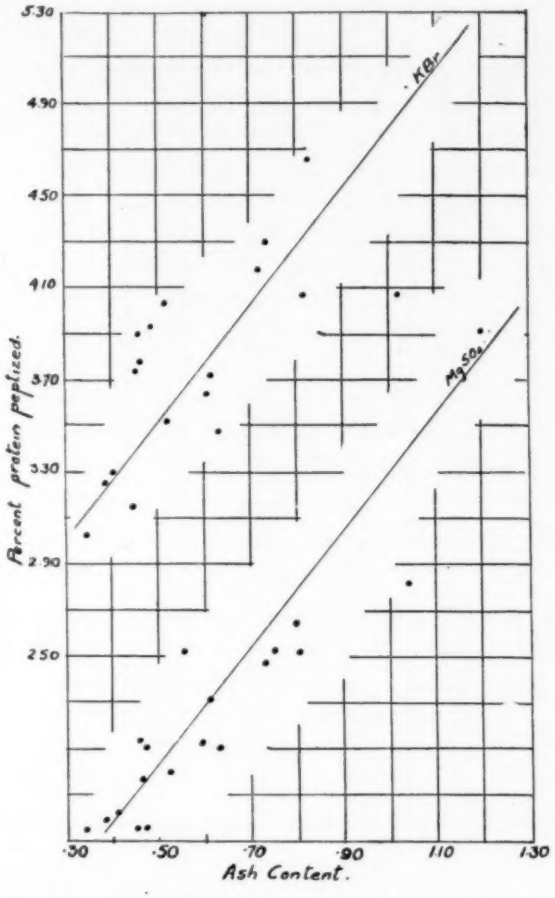

Fig. 3. Ash content of mill streams and amount of protein peptized by $MgSO_4$ and KBr solutions (from data of Harris, Cereal Chem. 8: 113–133).

In the above examples, as in the data published by Harris (1930 b), the percentage of protein peptized by $MgSO_4$ solution was very highly correlated with the ash content of the samples.

Discussion

For the experimentally milled flours of Geddes and Goulden (1930) and for those of Harris (1930) the variation in the amount of peptized protein is small. Although no ash content data are given, it is probably true that the variation in ash content was also very small. There is

TABLE V

PROTEIN PEPTIZED BY N/1 $MgSO_4$ SOLUTION

Series A—1930 crop			Series B—1930 crop			Series A—1931 crop		
Ash content	Total protein	Peptized protein	Ash content	Total protein	Peptized protein	Ash content	Total protein	Peptized protein
P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
0.34	11.20	1.48	0.35	12.20	1.64	0.34	11.70	1.96
.34	12.00	1.76	.38	12.30	1.64	.34	11.60	1.92
.35	11.40	1.84	.38	12.40	1.71	.34	11.80	1.96
.35	11.50	1.56	.39	12.10	1.60	.35	11.60	1.92
.35	12.10	1.48	.43	12.00	1.75	.37	11.70	1.92
.40	11.30	1.84	.43	12.70	1.71	.40	14.30	2.15
.40	11.30	1.83	.44	12.10	1.72	.42	11.50	2.27
.40	11.70	1.72	.47	13.70	1.88	.42	16.00	2.27
.41	11.50	1.64	.49	16.90	2.19	.45	12.70	2.27
.41	11.70	1.86	.52	11.90	2.00	.45	11.60	2.23
.41	13.80	1.88	.55	12.70	2.24	.46	11.90	2.35
.41	15.30	1.92	.57	14.20	2.12	.46	12.70	2.35
.44	12.50	1.88	.60	16.10	2.20	.46	14.10	2.15
.45	11.30	1.79	.61	12.50	2.31	.47	12.20	2.43
.46	14.50	1.88	.61	13.20	3.03	.51	13.30	2.55
.49	11.60	2.19	.62	12.70	2.64	.53	11.30	2.47
.49	12.90	1.88	.65	15.80	2.31	.55	14.70	2.39
.51	14.60	1.95	.69	13.40	2.43	.57	14.20	2.55
.52	12.00	2.07	.73	21.20	2.75	.58	13.60	2.75
.52	13.90	1.79	.75	14.80	2.47	.59	13.00	2.55
.53	13.70	2.03	.77	13.70	2.62	.59	11.30	2.67
.54	14.40	2.15	.78	13.10	2.59	.67	14.80	3.11
.58	13.00	2.19	.80	14.30	2.63	.78	13.10	3.39
.59	12.70	2.23	.97	17.70	2.87	.90	15.50	3.83
.65	15.00	2.71	1.16	23.10	3.59	.95	18.00	3.67
.66	13.80	2.19	1.20	14.80	4.11	1.15	17.20	3.39
.66	14.30	2.55	1.32	15.20	4.11	1.16	16.80	4.27
.70	13.00	2.43	1.65	15.50	4.59			
.71	13.10	2.55	1.65	17.50	4.75			
.76	18.80	2.55	1.78	19.80	4.87			
.77	14.80	2.63	2.10	17.70	6.94			
.82	14.90	2.79	2.30	19.60	6.50			
.97	17.30	2.95						

TABLE VI

CORRELATION BETWEEN ASH CONTENT OF MILL STREAM FLOURS AND THE AMOUNT OF PROTEIN PEPTIZED BY N/1 $MgSO_4$ SOLUTION

Series	r_{xy}	Value of r_{xy} at the 5% point
Mill A, 1930	+0.933	
Mill B, 1930	+0.937	+0.349
Mill A, 1931	+0.996	

always a small variation in the ash content of experimentally milled straight grade flours, probably enough to account for the small variation in peptized protein that occurred in their data.

The mill stream flours gave higher coefficients of correlation than the commercially milled flours. The former were naturally from the

same type of wheat. Examination of the source of the latter shows that almost every type of wheat grown in the United States was represented in the series used by Gortner, Hoffman, and Sinclair (1929). While the series used by Harris (1930) contained mostly Western Canadian hard spring wheat flours, they were milled from wheat from at least three different crops. The study of the hydration capacity of wheat flour proteins, made by Rich (1932), indicated that differences in that characteristic were present when wheats were classified according to variety, commercial wheat grade, and crop year. It seems probable that the peptizability of the proteins may also be different in different types of wheat, as represented by the above classification. If this is true, it would explain why correlation coefficients in the region of $+0.950$ were obtained with mill stream flours, and coefficients approximating (with $MgSO_4$) only $+0.750$ were obtained from series made up of different types of wheats.

Referring to Figure 4, it will be seen that the values for the 1930 and the 1931 crops lie at different angles to the axis of the graph, and at different levels. The equations for the two regression lines for the series of the two crop years are:

$$\text{For the 1930 crop } y = 2.00 + 2.3\ (x - 0.50)$$
$$\text{For the 1931 crop } y = 2.40 + 3.0\ (x - 0.50)$$

y represents the percentage of peptized protein and x the ash content. The differences between the proteins of the two years occurred both in the amount peptized and in the rate of peptizability with increasing ash content. It is evident that the inherent peptizability of the proteins was different for the two years studied.

The data of Geddes and Goulden (1930) indicate that the peptizability of the proteins is not changed when the wheat has been damaged by frost.

No study of the peptizability of the proteins of wheats of different varieties was attempted at this time, nor were any data found in the available literature.

It is very probably true that the ash constituents of flour are not in themselves responsible for differences in the peptizability of wheat flour proteins. It is difficult to conceive how such relatively small amounts of ash constituents could have any measurable influence on peptizability in the presence of such relatively large amounts of electrolyte in the extracting medium. It is, however, very probable that ash content of flour is very highly correlated with the causal factor influencing peptizability. If this is true, then, *most* of the differences in the peptizability of flours are not due to any inherent differences caused by environmental or genetical factors, but are due to some factor which varies

according to the length of the patent of the flour or to the streams making up the sample.

The lower grades of flour (which are highest in ash content) are undoubtly more contaminated with germ than those from the purer

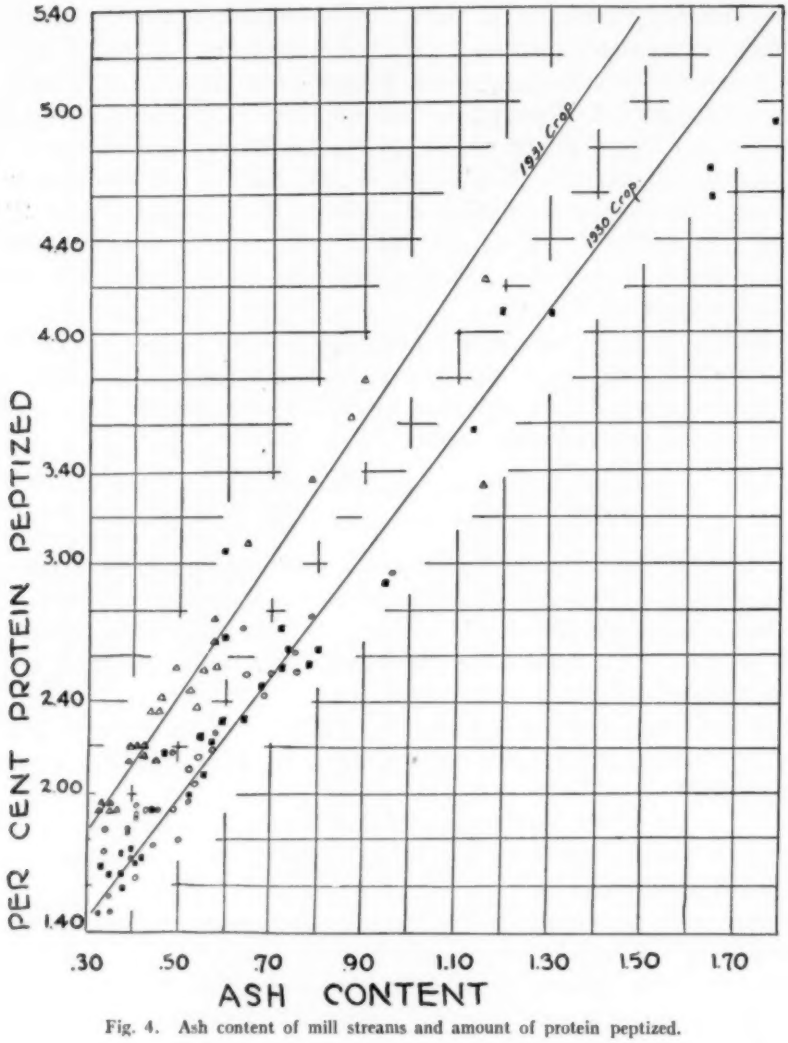

Fig. 4. Ash content of mill streams and amount of protein peptized.

middlings streams. The investigations of Working (1924, 1928) and Geddes (1930) on the influence of phosphatides on flour properties suggest that these constituents may be the causal factor for the increase in the peptizability of the proteins with decrease of the grade (i.e., increase of the ash content) of the flour.

Gortner (1927), Gortner, Hoffman, and Sinclair (1929), Geddes and Goulden (1930), and Harris (1930) have attempted to demonstrate a relationship between the peptizability of wheat flour proteins and baking strength. In the words of Geddes and Goulden (1930), "it appears difficult to correlate and interpret the data thus far published on peptization." For experimentally milled flours, since the range of protein peptized is narrow, the correlation between non-peptized protein and loaf volume is almost as high as that between protein and loaf volume. It must be remembered that non-peptized protein is the difference between total and peptized protein, and that in every case the magnitude of the correlation is influenced by the magnitude between total protein and loaf volume. Examination of the tables of any of the data indicates that the per cent of total protein peptized is independent of the total protein content. Much of the discussion thus far published has dealt with this value. Geddes and Goulden (1930) point out that because the per cent of total protein peptized is the quotient of per cent of peptized protein and total protein, then the magnitude of the correlation of per cent total protein peptized and loaf volume is due to the variation in total protein and, therefore, cannot be used as a measure of any real value.

If the amount of protein peptized by a given salt solution is highly correlated with the grade of the flour, as measured by its ash content, then it is not of any greater value in predicting baking strength than is ash content alone. It has been shown, however, that when the influence of ash content is eliminated by plotting ash content against the per cent of peptized protein, that small differences did occur between the flours of the 1930 and 1931 crops. The data of Gortner, Hoffman, and Sinclair (1929) indicate that small differences may occur between wheats of different types.

Conclusions

The per cent of wheat flour proteins peptized by a solution of a neutral salt of a strong acid and a strong base is very highly and positively correlated with the ash content of the sample.

Most of the differences in the peptizability of wheat flour proteins are due to some factor which is very highly correlated with the ash content of the flour.

Relatively small differences in the inherent peptizability of the proteins from flours of the Western Canadian crops of 1930 and 1931 were found. The differences occurred both in the amount peptized by N/1 $MgSO_4$ solution and in the rate with increasing ash content.

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EFFECT OF NUMBERS OF BACTERIA ON THE DEVELOPMENT OF RANCIDITY IN SOFT WHEAT FLOUR[1]

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Introduction

Examination of the literature shows that a number of investigators have noted the occurrence of micro-organisms in flour. Papasotirin (1902) reported the presence of *B. coli* in all white and black bread flours and upon the seeds of grains. Scott (1920) noted that organisms of the *B. mesentericus* group were responsible for ropy bread. Kuhl (1911) reported the presence of *B. subtilis* in all flours. Thom and LeFevre (1921) in analyzing several samples of corn bread noted that on the average the meal contained from 50,000 to 70,000 bacteria per gram. Fred (1929) analyzed bacteriologically 5 lots of flour and found the bacterial content of the flour to be from 18,000 to 60,000 per gram of moist flour. Data on this subject have also been contributed by Kent-Jones and Amos (1930).

The object of this experiment was to make an intensive study of the influence of total numbers of bacteria on the development of rancidity in soft wheat flours.

Methods

Determination of Odor. Fifty grams of flour were extracted with 150 cc. of ethyl ether free from peroxides for 1 hour or longer. The extract was then carefully decanted through a Whatman No. 3 filter paper. The ether was then evaporated slowly on a steam bath and the "*odor*" noted.

Spectroscopic Examination of the Kreis Test. After noting the odor of the oil, 5 cc. of concentrated HCl was added to the oil and mixed for 30 seconds. Fifteen cc. of a 0.1% solution of phloroglucinol in ether was then added and the mixture again shaken for 30 seconds. The mixture was transferred to a 1 × 4-inch test tube and the color of

[1] These data are from a thesis submitted by C. B. Gustafson to the Graduate School of Purdue University in partial fulfillment of the requirements of the degree of Master of Science, June, 1932. This work was supported by a Fellowship from The National Soft Wheat Millers' Association.

the acid layer noted in 10 minutes. The color was examined spectroscopically for the presence or absence of an absorption band in the yellow green region of the spectrum. Rancid oils and fats show a definite absorption band while non-rancid ones do not. Powick (1923) has shown that the position of the absorption band is identical with that obtained in the acrolein-hydrogen peroxide test.

Bacteriological Tests. The technic used for the enumeration of the bacterial content was similar to that of Kent-Jones and Amos (1930). Standard nutrient agar (American Public Health Association, 1927) was found to yield as high counts as any of the media compared. The media compared were standard nutrient agar, peptonized milk agar, yeast extract agar, yeast extract dextrose agar, potato dextrose agar, and beef infusion agar.

Incubation of plates was 5 days at 25° C., then 1 day at 37° C. Dilutions used were 1 to 1,000 and 1 to 10,000. Because of flour specks, dilutions of 1 to 100 were difficult to read, and results could not be relied upon. Plates were all made in duplicate and the arithmetic average recorded.

Material

Samples of all grades of flour together with samples of wheat from which these flours were milled were obtained from 19 mills representative of the soft red winter wheat production area.

The wheat samples were milled experimentally into a straight grade flour which later was bleached and used in a comparative study with those milled commercially. Before milling the first sample of wheat in the experimental mill, 5, 2-pound samples of sterilized wheat were passed through the mill. The number of bacteria remaining in the mill after such treatment was very low as determined by a preliminary milling test. The same procedure was used before the milling of each sample of wheat to prevent contamination of one sample with another.

After samples had been taken for bacteriological analysis each flour sample was divided into two parts of about 1,000 gms. each. One part was made into self-rising flour using the following formula: Flour 95.03%, phosphate 1.80%, soda 1.42%, salt 1.75%, total 100%.

The samples, both plain and self-rising, were then transferred to 3-pound cotton flour sacks and stored at a temperature of from 70° to 80° F. The sacks of flour were spaced about 6 inches apart on the shelves so that all samples were equally exposed to air.

Experimental Data

Table I shows that the wheat had a higher bacterial count than the flour made from it. Of the flours the best grade (patent) had on the

average a lower count than the poorer grades (clear). The experimentally milled flours likewise had a higher bacterial count than the commercially milled flours.

TABLE I

COMPARISON OF BACTERIAL COUNTS OF WHEAT AND FLOURS MADE AT THE BEGINNING OF THE STORAGE PERIOD

	Wheat	Patent	Straight	Clear	Experimentally milled (Straight)
Number of samples	19	20	17	18	16
Maximum count per gram	3,260,000	146,000	180,000	380,000	880,000
Minimum count per gram	46,000	9,000	30,000	30,000	54,000
Geometric mean	394,000	60,700	72,500	87,600	154,000

In Tables II to V are shown the rancidity observations and bacterial count for the patent, straight, clear, and experimentally milled flours studied. Table VI is a summary of the data for all the samples studied. It will be noted that the negative groups often include samples of very high bacterial count, while the positive rancid groups frequently include samples of low bacterial count.

Considering the geometric mean of the bacterial counts, there is shown no consistency in results in regards to rancidity, and in most cases the difference in bacterial count, between the samples developing rancidity and those not, is within the limits of experimental error of bacterial plate counting.

TABLE II

COMPARISON OF THE NEGATIVE AND POSITIVE RANCID PATENT FLOURS WITH THE BACTERIAL COUNT

Days of storage	Number of samples	Test	Result	Bacterial count per gram		
				Geometric mean	Maximum	Minimum
55	35	odor	neg.[2]	61,000	145,000	9,000
	36	spect.[1]	neg.	61,000	145,000	9,000
	3	odor	pos.[3]	22,000	35,000	9,000
	2	spect.	pos.	35,000	35,000	35,000
80	34	odor	neg.	60,000	145,000	9,000
	29	spect.	neg.	61,000	145,000	9,000
	4	odor	pos.	48,000	94,000	25,000
	9	spect.	pos.	54,000	113,000	13,000
143	32	odor	neg.	57,000	145,000	9,000
	28	spect.	neg.	61,000	145,000	9,000
	6	odor	pos.	65,000	118,000	25,000
	10	spect.	pos.	54,000	124,000	13,000

[1] Spectroscopic.
[2] Negative.
[3] Positive.

TABLE III

COMPARISON OF THE NEGATIVE AND POSITIVE RANCID STRAIGHT FLOURS WITH THE BACTERIAL COUNT

Days of storage	Number of samples	Test	Result	Bacterial count per gram		
				Geometric mean	Maximum	Minimum
55	32	odor	neg.	78,000	180,000	28,000
	33	spect.	neg.	75,000	180,000	28,000
	4	odor	pos.	50,000	54,000	47,000
	3	spect.	pos.	67,000	104,000	54,000
80	29	odor	neg.	80,000	180,000	28,000
	23	spect.	neg.	80,000	180,000	28,000
	7	odor	pos.	55,000	104,000	46,000
	13	spect.	pos.	64,000	118,000	46,000
143	29	odor	neg.	80,000	180,000	28,000
	22	spect.	neg.	78,000	180,000	28,000
	7	odor	pos.	55,000	110,000	46,000
	14	spect.	pos.	69,000	118,000	47,000

TABLE IV

COMPARISON OF THE NEGATIVE AND POSITIVE RANCID CLEAR FLOURS WITH THE BACTERIAL COUNT

Days of storage	Number of samples	Test	Result	Bacterial count per gram		
				Geometric mean	Maximum	Minimum
55	30	odor	neg.	82,000	380,000	30,000
	25	spect.	neg.	86,000	380,000	30,000
	6	odor	pos.	120,000	173,000	59,000
	11	spect.	pos.	91,000	173,000	30,000
80	18	odor	neg.	101,000	380,000	30,000
	15	spect.	neg.	112,000	380,000	30,000
	18	odor	pos.	76,000	173,000	30,000
	21	spect.	pos.	74,000	173,000	30,000
143	16	odor	neg.	84,000	209,000	30,000
	12	spect.	neg.	102,000	209,000	81,000
	20	odor	pos.	91,000	380,000	30,000
	24	spect.	pos.	81,000	350,000	30,000

In Table V, in which all samples are grouped together depending on their development of rancidity, it appears that those samples of flour which did develop rancidity had a higher geometric mean than those samples which did not. However, it is to be noted in Table IV, which contains the bacterial counts on the experimentally milled flours, that these flours showed from 2½ to 3 times greater count than did the commercially milled flours, and that a larger number of the experimentally milled samples became rancid and thus affected the geometric mean

TABLE V

COMPARISON OF THE NEGATIVE AND POSITIVE RANCID EXPERIMENTALLY MILLED FLOURS WITH THE BACTERIAL COUNT

Days of storage	Number of samples	Test	Result	Bacterial count per gram		
				Geometric mean	Maximum	Minimum
55	22	odor	neg.	153,000	910,000	57,000
	14	spect.	neg.	151,000	910,000	54,000
	10	odor	pos.	158,000	880,000	54,000
	18	spect.	pos.	157,000	880,000	54,000
80	5	odor	neg.	216,000	910,000	57,000
	4	spect.	neg.	202,000	910,000	112,000
	27	odor	pos.	144,000	880,000	54,000
	28	spect.	pos.	149,000	880,000	54,000
143	6	odor	neg.	180,000	910,000	57,000
	3	spect.	neg.	72,000	112,000	57,000
	26	odor	pos.	149,000	880,000	54,000
	29	spect.	pos.	167,000	910,000	54,000

TABLE VI

COMPARISON OF ALL NEGATIVE AND POSITIVE RANCID FLOURS WITH THE BACTERIAL COUNT

Days of storage	Number of samples	Test	Result	Bacterial count per gram		
				Geometric mean	Maximum	Minimum
55	119	odor	neg.	82,000	910,000	9,000
	108	spect.	neg.	80,000	910,000	9,000
	23	odor	pos.	100,000	880,000	9,000
	34	spect.	pos.	109,000	880,000	30,000
80	86	odor	neg.	80,000	910,000	9,000
	71	spect.	neg.	84,000	910,000	9,000
	56	odor	pos.	96,000	880,000	25,000
	71	spect.	pos.	92,000	910,000	13,000
143	83	odor	neg.	77,000	910,000	9,000
	65	spect.	neg.	74,000	209,000	9,000
	59	odor	pos.	101,000	880,000	25,000
	77	spect.	pos.	97,000	910,000	13,000

of all samples showing rancidity more than the geometric mean of the samples not showing rancidity.

At the beginning of the experiment the plain and self-rising flours had the same bacterial counts except for the few bacteria which may have been added by the introduction of self-rising ingredients. At the conclusion of the storage period of 143 days, as shown in Table VIII, the difference in bacterial counts of the plain and self-rising flours is within the limits of error of bacterial count.

TABLE VII

COMPARISON OF THE BACTERIAL COUNTS OF PLAIN AND SELF-RISING FLOURS AFTER 143 DAYS STORAGE

	Patent		Straight		Clear		Experimentally milled	
	Plain	S.R.[1]	Plain	S.R.	Plain	S.R.	Plain	S.R.
Number of samples	20	20	17	17	18	18	16	16
Maximum count per gram	170,000	259,000	140,000	119,000	220,000	210,000	1,000,000	1,070,000
Minimum count per gram	12,000	7,000	12,000	16,000	18,000	20,000	46,000	27,000
Geometric mean	51,400	51,100	50,700	43,200	69,500	67,700	144,000	163,000

[1] Self-rising.

Summary

1. The total bacterial count of wheat was greater than that of the flour made from it.

2. The bacterial count of flour tends to decrease with time of storage.

3. Self-rising and plain flours had practically the same bacterial count.

4. With the flours studied, the total bacterial count, of self-rising or plain flour, did not appear to be a dominant factor in the development of rancidity.

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THE SITOSTEROL ESTERS IN WHEAT FLOUR OIL

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A crystalline substance, believed to be an ester of sitosterol, has been isolated from the ether extracts of wheat flour by Ritthausen (1863), Gortner (1908) and Ball (1926). Winterstein and Smolenski (1909) found the same material in concentrated alcoholic extracts after dilution with ether. Walde and Mangels (1930) found it in the acetone extract of common wheat flour but not in that of durum flour. Martin and Whitcomb (1932) observed the substance in ether extracts of Marquis flour, but it separated from Kubanka flour extracts only on cooling to — 16° C.

Winterstein and Smolenski's preparation melted at 91–92° C. and on saponification gave a sterol which melted at 124° C. Gortner reported the melting point as 96.5° C. which corresponds to the top fractions obtained by Ball after several crystallizations. The latter's compound saponified to give a sterol which melted at 137.5° C. and whose acetate melted at 125.5° to 126.0° C. The ester isolated by Walde and Mangels melted at 93° C. and gave a sterol melting at 137° C.

The properties are those of a sitosterol ester, but in view of the complexity of natural sitosterols (Anderson, Shriner, and Burr, 1926) and the fact that the acid part has not been isolated, the characterization of the compound cannot be considered entirely adequate.

In the present work the ester was prepared from the acetone extract of a sample of unbleached patent flour in much the manner described by Walde and Mangels. The melting point was 93–94° C. After saponification with alcoholic alkali the acid part was isolated and found to be pure palmitic acid as characterized by the melting point, analysis, neutralization equivalent, and melting point of the *p*-bromophenacyl ester. The sterol part melted at 136.5° C. and had the rotation $[\alpha]_D$ — 13.3°. The acetate melted at 125.5° C. Ritter (1901) synthesized a sitosterol palmitate and reported the melting point as 90° C. The sitosterol which he used melted at 136.5° C. and had the rotation $[\alpha]_D$ —33.9°.

The melting point of a sitosterol means but little; however, the weak laevorotation of the sterol isolated in this work made it certain that

a mixture was present. Pure γ-sitosterol melts above 142° C. (Bonstedt, 1928) and the specific rotation is —42° to —46°. Rotations of α- and β-sitosterol are not known although they are much lower, perhaps —25° to —35°. On the other hand, dihydrositosterol which occurs in the wheat endosperm (Anderson and Nabenhauer, 1924) is *dextro*rotatory; the values commonly reported vary from 25° to 28° C. A sitosterol preparation of $[\alpha]$ —13.3° must therefore contain a rather large fraction of dihydrositosterol. This cannot be calculated exactly from the rotation alone, but it would appear to be roughly one-third. A better measure of the amount of dihydrositosterol was found in a quantitative estimation of unsaturation. Methods involving the halogens were not feasible on account of the tendency of α-sitosterol to halogenate by substitution. The use of camphoric acid peracid recently introduced as a modification of the Prileschajew reagent by Milas and Cliff (1933) has been shown to be reliable in the sterol series and consequently seemed suited to the present problem. Results were concordant, but in calculating the amount of available oxygen used as per cent of the theoretical value a choice had to be made as to the empirical formula of sitosterol. It has long been written $C_{27}H_{46}O$, but recently Sandqvist and Bengtsson (1931) and Windaus, Werder, and Gschaider (1932) have changed this to $C_{29}H_{50}O$. The camphor peracid titration gave values which on the basis of the C_{29} formula indicate the presence of 28.3% and 28.8% of dihydrositosterol. The use of the C_{27} formula leads to the somewhat higher figures 33.2% and 33.6%. Either set of data indicate clearly that the sitosterol palmitates of wheat flour are derived from a mixture of sterols composed of slightly less than one-third of dihydrositosterol and somewhat more than two-thirds of sitosterols which, in view of their low rotatory power, must be rich in the α and β modifications.

Experimental

The starting material was a sample of unbleached patent flour milled from Marquis wheat grown in North Dakota. Lots of 1.3 kg. were extracted continuously with acetone for 48 hours. The extract was concentrated to 400 cc. and cooled in an ice-salt bath for several hours. The waxy precipitate was separated by decantation and washed with successive small portions of cold acetone until the washings were colorless. After purification by crystallization from alcohol the ester appeared as nacreous, matted platelets which melted at 93° to 94° C. Yields were consistently 0.4 gm.

Analysis. Calculated for sitosterol palmitate, $C_{45}H_{80}O_2$: C, 82.7; H, 12.3. Found: C, 82.7; H, 12.4.

Saponification was achieved by boiling 0.74 gm. of the substance for 7 hours with 25 cc. of 10% potassium hydroxide in aldehyde-free alcohol. The mixture was poured into water and extracted several times with ether to collect the sterols. The ether extract was washed with water and the washings were added to the alkali. This was acidified and extracted six times with ether to recover the acid part of the ester.

The acid (0.15 gm.) crystallized from methanol as colorless leaflets; m. p. 62.5° C.

Analysis. Calculated for palmitic acid, $C_{16}H_{32}O_2$: Neutralization equivalent, 256; C, 75.0; H, 12.5. Found: Neutralization equivalent, 257; C, 75.1; H, 12.2.

The *p*-bromophenacyl ester was prepared and found to melt at 86°. Hann, Reid, and Jamieson (1930) report 86°.

The sterol part was recovered after evaporation of the ether solution and crystallized twice from alcohol. It weighed 0.31 gm. m. p. 136.5° C. (uncorr.); $[\alpha]_D$ — 13.3° (c = 1.13 in chloroform). It was desiccated in a vacuum oven at 115° C. and analyzed.

Analysis. Calculated for sitosterol, $C_{29}H_{50}O$: C, 83.98; H, 12.16. Calculated for dihydrositosterol, $C_{29}H_{52}O$: C, 83.57; H, 12.59. Found: C, 83.54, 83.67; H, 12.1, 12.0.

The camphoric acid peracid titrations were carried out as directed by Milas and Cliff (1933). Samples of 77.1 and 68.9 mgs. required available oxygen equivalent to 5.07 cc. and 4.50 cc. of .05263N thiosulfate. In terms of the C_{29} formula these represent respectively 71.7% and 71.2% of the theoretical requirement, while for the C_{27} formula the values are 66.8% and 66.4%.

Summary

The sitosterol ester isolated by several investigators from wheat-flour oil has been shown to be a mixture of palmitates. The sterol part contains approximately 28% of dihydrositosterol and 72% of sitosterol which is believed to be rich in the α and β modifications.

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IN VITRO DIGESTION OF THE STARCH OF LONG AND SHORT COOKED CEREALS

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In a recent *in vitro* study on the digestibility of the starch of cereals, carried out by the authors, it was found that digestion, as shown by the amount of sugar produced with diastase, was as complete in the cereals cooked 20 minutes as in those cooked 90 minutes. This finding is in disagreement with a part of the results of Carman, Smith, Havens, and Murlin (1929) who report that cereals which had not been cooked as a part of their manufacture were less completely digested (by saliva) when boiled for a short period (30 minutes) than for a long period (60 minutes). It is, however, in agreement with the *in vivo* studies of Rose (1922) and of Kramer and Halstead (1925) on the digestibility of the starch of rolled oats and corn meal.

The cereals used for the present study were two pre-cooked cereals and five cereals which had been heated only enough to sterilize the grain. The former were toasted whole wheat and rolled oats; the latter a whole wheat product and cereals made from the endosperm of wheat, white corn, yellow corn, and barley. All were granular except the oats. They were cooked in the quantity necessary to yield approximately 2 cups when finished. The amount of water was adjusted to give porridges of approximately like stiffness and of a consistency acceptable in a breakfast cereal. The water was brought to the boiling point, the cereal sifted in and the mixture allowed to boil over a flame for 2 minutes after which the container was placed in the lower part of a double boiler and the cereal allowed to cook for the required time. Samples for analysis were removed after 20 and 90 minutes.

A raw and the two cooked samples of each cereal were digested for an hour with malt diastase in an oven at 38° C. The oven was equipped with an electrically driven stirring device which kept the cereal and enzyme solution well mixed. The action of the diastase was checked and the protein and undigested residue precipitated by Rumsey's method (1923) of adding sodium tungstate and acid to give a pH value of approximately 2. After centrifuging, the clear supernatant liquid was analyzed by the Munson-Walker thiosulfate method.

The results are given in the table which follows. Each figure represents an average obtained from three or more separate cookings with at least duplicates on each cooking. To be significant a difference between two results must be larger than 5 since in one or two cases independent cookings of the same cereal showed a variation of this magnitude when computed on the basis of 100 gms. of dry cereal. As will be seen from the table, and as already mentioned, there is a striking similarity of the values for the 20 and 90-minute cooking periods for all of the cereals whether they be pre-cooked or not, whether they be granular or rolled, or whether they consist of the endosperm only or of the whole grain. Incidentally it will be seen that the raw cereals show much lower figures than the cooked samples. This indicates that, although raw cereal starches may be fully digested as was found to be true in the *in vivo* experiments already mentioned, they are not converted into sugar as readily as when cooked.

MALTOSE FORMED BY THE ACTION OF DIASTASE ON CEREALS

Cereal	Maltose [1] formed by 1 hour digestion of: Uncooked cereal	Cereal cooked 20 min.	Cereal cooked 90 min.
	Gms.	*Gms.*	*Gms.*
Toasted whole wheat	15.05	61.19	61.51
Rolled oats	35.14	51.53	46.51
Whole wheat	4.57	53.16	54.72
Farina I	5.68	62.66	62.20
Farina II	5.44	68.81	68.29
Corn meal (white)	22.92	66.18	63.85
Corn meal (yellow)	20.10	61.53	58.87
Barley endosperm	12.85	58.20	56.08

[1] Computed on 100 gms. dry cereal.

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A SIMPLE VOLUMETRIC METHOD FOR MEASURING GAS PRODUCTION DURING DOUGH FERMENTATION

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In bread making, gas production during dough fermentation, which is a matter of the greatest importance, depends both on flour properties and the capacity of the yeast.

Almost universally, the fermenting power of yeast has been determined by measuring gas production in a sugar solution. In different text-books mention is made of nothing but this method, although it is a well-known fact that, in view of baking practice, the carbon dioxide produced during the fermentation of the dough is a much better criterion (Elion and Elion, 1929).

Hayduck (1882) described a simple gas burette for the determination of carbonic acid, evolved during alcoholic fermentation of a sugar solution, which consists of a calibrated measuring tube, connected at its bottom with a water container. The gas is introduced at the top of the burette and, for determining its volume, the water container is raised or lowered so as to bring the water in the container and in the measuring tube to the same level. The gas volume results from the difference of two readings.

Kusserow (1897) used a modified apparatus, consisting of a water displacement gasometer (Fig. 1). The gas, evolved by a fermenting sugar solution in bottle *A*, is introduced into a spherical vessel *B*, filled with an appropriate liquid, which is sent over by the gas into a graduated cylinder and measured.

Kunis (1901), instead of using the spherical vessel *B*, made use of a simple flask with a 2-hole cork stopper (Fig. 2). In this form the apparatus may be easily constructed in every laboratory but it has the drawback in that gas pressure is not maintained constant, and, therefore, the water volume measured is not the same as the volume of the gas produced. This error, which increases with the height of flask *B*, may easily amount to several per cent. Kent-Jones (1927), Jørgensen (1931), and Markley and Bailey (1932) associated with this apparatus the name of Jago (1911). Jago indeed used the same method, but omitted to mention that it was described for the first time by Kusserow

(1897) and by Kunis (1901). James and Huber (1928) also mentioned the use of Kusserow's method in conducting their experiments.

H. Elion (1903) used an apparatus similar to Hayduck's gas burette

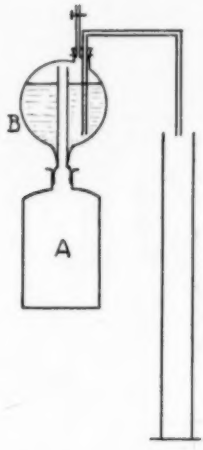

Fig. 1. Apparatus of R. Kusserow.

for the purpose of measuring gas production during *dough* fermentation. This method is a very easy one to use if the quantity of carbonic acid does not exceed several hundreds of cubic centimeters. With

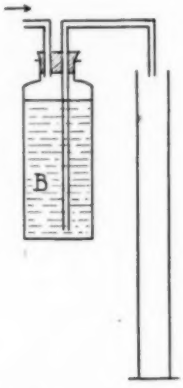

Fig. 2. Apparatus of K. Kunis.

larger volumes the manipulation of the water container becomes more difficult, because of the weight of the quantity of water required.

The principle of a water displacement gasometer for determining gas production during dough fermentation is very simple indeed, but,

as far as the author knows, no apparatus has been described which permits the measurement of gas production at constant pressure and in which, at the same time, the volume of the liquid displaced and measured is exactly equal to the gas volume.

Markley and Bailey (1932) recently described an interesting automatic water displacement gasometer which permits the maintenance of a constant gas pressure. The water measured, however, is proportional but not equal to the air displaced by the fermenting dough, and the apparatus must be calibrated by passing known quantities of air or water into the fermentation jar. These authors used a siphon, fastened to a balanced float, for the purpose of maintaining atmospheric pressure in the fermentation jar. The principle of a floating siphon originated with Waller (1896), of Delft, Holland, for collecting gases in a very regular and automatic manner for analytical purposes.

A simple water displacement gasometer, which may be easily constructed in every laboratory and enables one to maintain a constant gas pressure during all readings, in which, moreover, the measured water volume is equal to the gas produced, is diagrammed in Figure 3. The

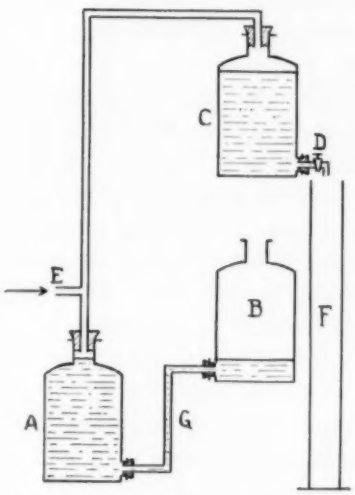

Fig. 3. Water displacement gasometer.

bottles *A*, *B*, and *C*, with tubulure near the bottom, contain an appropriate indifferent liquid or water, overlaid in *A* and *C* with a film of an indifferent liquid. The bottles *A* and *B* are placed in such a way, that, when the liquid in *A* reaches into the neck of the bottle, a little under the cork, the level in *B* is somewhat above the tubulure. The tube of stopcock *D* is filled with the liquid.

At the beginning, there is atmospheric pressure in the whole apparatus and the liquid in *A* reaches into the neck and to the same level as in *B*. The fermentation jar is then connected with *E* and, the stopcock *D* being closed, the liquid in *A* is displaced into the bottle *B*. Of course *A* must be large enough to prevent any loss of gas through its tubulure near the bottom.

If one wants to measure the gas volume produced during a definite period, the only thing to be done is the opening of the stopcock *D*, through which liquid is sent over into the graduated cylinder *F*. At the same time liquid from *B* returns into *A* and the stopcock *D* must be closed as soon as the levels in *A* and *B* arrive at their original position. At that moment the gas in the apparatus again has atmospheric pressure and the quantity of water in the graduated cylinder is exactly equal to the volume of carbonic acid produced. If desired, these values may be reduced by calculation to normal conditions.

In view of the accuracy of the method, it is desirable that the liquid in *A* have a small surface at the moment of the readings, because, otherwise, experimental errors become too great.

The flow of the water from *C* into *F* may be regulated so as to maintain approximate atmospheric pressure in the apparatus during the whole experiment. However, the passing of too much water to the graduated cylinder has to be prevented. The bottle *C* may be large enough to suffice for more than one determination, and the liquid displaced from *C* may be used again for the next measurement.

It is, of course, not necessary for connecting the bottoms of *A* and *B*, to make use of bottles with a tubulure. Ordinary flasks with 2-holed corks may be employed just as well, the desired connection being obtained by a simple tube reaching to the bottom in both bottles and remaining permanently filled with liquid. A device of the same kind is possible for emptying the bottle *C*, so that even here an ordinary flask may suffice.

Preferably tube *G* is a glass one, so as to prevent liquid remaining in a rubber tubing, which, as in the case of Hayduck's gas burette, often causes leakage or even rupture of the tubing.

Summary

Water displacement gasometers, previously described, have the drawback that either gas pressure is not maintained constant during the readings or the water volume measured is not equal to the gas produced. A simple water displacement gasometer for measuring gas production during dough fermentation is described, which may be easily constructed in every laboratory and enables one to maintain a constant

gas pressure during all readings, in which, moreover, the measured water volume is equal to the gas produced.

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THE WHEAT-MEAL FERMENTATION TIME TEST OF "QUALITY" IN WHEAT AS ADAPTED FOR SMALL PLANT BREEDING SAMPLES [1]

G. H. CUTLER and W. W. WORZELLA [2]

(Received for publication April 15, 1933)

In a preliminary report Cutler and Worzella (1931) have described a method for measuring "quality" in wheat. Supporting data resulting from a large number of experimental tests were presented. During the past two seasons further studies have been conducted. These have shown in an even more conclusive manner the validity of the relationships of "time" as an index of gluten strength in wheat.

This method has been designated the wheat meal fermentation time test.[3] It has been employed in measuring "quality" in wheat in the Agronomy Department for the past four years. Not only has it been used in the soft wheat breeding program in selecting strains of desirable milling and baking qualities, but all standard varieties, whether grown at the outlying stations or at Lafayette, have been subjected to it. Furthermore, it has been used in studying miller's samples in an attempt to ascertain miller and baker requirements in soft wheats. Specific investigations have also been undertaken to further study and perfect the technique of the test. This paper will briefly report the recommended procedure and present some of the results obtained from these studies.

The Method

In our original paper emphasis was given to the importance of careful attention to details of procedure. Further studies and experience have served to re-emphasize the wisdom of this statement. Sound, dry, clean, normal wheat is recommended in making the test. It has been found that scabby, weevily wheat or wheat treated with disinfectants do not respond satisfactorily. Furthermore, meal that contains too much moisture makes up into rather soft, wet dough-balls when the standard amount of yeast solution is used, and, as a consequence, the "time" is considerably changed.

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[2] Assistant Chief and Professor of Agronomy, and Assistant in Agronomy and Fellow of the National Milling Company, respectively.

[3] Other terms used interchangeably in this paper for the official designation are—fermentation test and time test respectively.

It is also recommended that a standard wheat of known "time" be tested each day to serve as a check or control. Such a practice will be found helpful in detecting variations which might arise from day to day.

Age of wheat when tested. Since wheat continues to improve in "quality" for some time after harvesting, the authors follow the practice of testing for "quality" any time after six weeks following wheat harvest. It has been found too that the "quality" of ground wheat improved more rapidly than unground wheat and that it varies to some extent with the variety of wheat. As a consequence grinding is deferred until the fermentation tests can be carried out. Accordingly, an attempt is made to grind in the afternoon what can be tested the following day. Usually 2 to 3 series of soft wheats can be run through in the morning and early afternoon and the balance of the day is given over to grinding samples.

Grinding the Wheat. Proper grinding of the wheat is essential. A power driven grinder known as the Wiley mill has been used at Purdue for grinding. This grinder enables one to secure the desired fineness of meal by means of a brass sieve of 1 mm. mesh that fits into the frame of the grinding chamber. Other grinders both hand and power driven were tested. The Labconco mill has proven quite satisfactory; it grinds rapidly, is easy to operate, cleans itself quickly and thoroughly and is equipped with adjustments that enable one to standardize its operation with that of the Wiley mill. If hand grinders are used much care must be exercised, especially when grinding the harder wheats. Whether using power or hand mills, one should take the precaution to standardize with the Wiley mill.

Making the yeast solution. A yeast solution is prepared by dissolving 10 gms. of Fleishmann's compressed yeast in 100 cc. of distilled water. A new solution should be made up each day from fresh yeast or from yeast kept in a refrigerator or ice box.

Making the dough-ball. When ready to proceed, carefully mix the wheat-meal to insure an even distribution of the finer and coarser particles. Weigh out triplicate 10-gm. samples and place each into a 150 cc. low form beaker and add 5.5 cc. of the yeast solution which is maintained at 80° F., and with a glass stirring rod mix into a mass. The mass is then transferred to the palm of the left hand and with the right thumb is kneaded (for about a minute) until it becomes a dough-ball of medium-stiff consistency. Since the moisture absorption varies with different wheat varieties and strains, some dough-balls will require additional water; hence water is added when needed (do not add more of the yeast solution) and followed by further kneading until the right

consistency is obtained. Finally the ball is rolled in the hands into a small sphere, free from folds and crevices.

The amount of meal required, or the size of the dough-ball is a matter that is of special interest to the wheat breeder and geneticist who is limited in the amount of seed available. The size of the dough-ball recommended as standard is 10 gms., but tests conducted during the past two seasons indicate that a 3.5- or 5-gm. dough-ball can be used with satisfactory results by proportionately reducing the amount of yeast solution, the size of beaker, and amount of water in the bath. Accordingly, with a 3.5-gm. sample, 2 cc. of the yeast solution is used and the dough-ball is placed in a 50 cc. beaker which contains 30 cc. of distilled water.

Measure of gluten quality. Each dough-ball is placed in a low form beaker containing the appropriate amount of distilled water (for a 10-gm. dough-ball 80 cc. of water is used) kept at 80° F. The time is recorded at once and the beakers are placed in a fermentation cabinet.

The dough-ball soon becomes buoyant and rises to the surface of the water where it increases in size—in soft wheats the form of the dough-ball is more or less spherical in outline, while in the harder wheats it becomes flat and tends to cover the surface of the water. Regular observations are made of all dough-balls to observe when disintegration begins. This is usually on the lower surface. When this takes place the time is again recorded. The "time" of each sample is thus obtained. It is recorded in minutes and consists in the difference between the original immersion and the first fall of detached dough. Thus "time" becomes the index or measure of quality.[4] The longer the time the stronger the gluten; this has been found to vary from as low as 15 minutes in some of the soft wheats to 400 or more minutes in the hard wheats. The wheats preferred for pastry flours have a short "time" test, while those preferred for bread flours have a long "time."

Variation in "time." A 10% variability rule has been observed as a basis of agreement among triplicate dough-ball tests. When satisfactory agreement is not obtained among triplicate tests, the entire test is repeated. It should be said that during the four years in which several thousand separate dough-balls have been made, it has seldom been found necessary to repeat a test.[5]

[4] Pelshenke (1930), of Halle, Germany, working simultaneously and independently, discovered a test for "quality" in wheat which is essentially the same. However, in addition to "time" as an index of gluten quality Pelshenke used "specific protein quality." This was arrived at by first determining the time and the per cent protein, respectively, of each sample and then dividing the time by the protein. The authors repeatedly find an almost perfect positive correlation ($r = +.996 \pm .0015$) between "time" and "specific protein quality" and therefore do not feel that the additional cost is warranted. Furthermore, it requires additional seed and is not so suitable for small plant breeding samples.

[5] The bulk of these tests has been made among the soft and semi-hard wheats, though much experimental work has also been carried out among the typical hard classes of wheat.

The exceptions, if they may be so termed, would seem to be associated with varieties of wheat which show wide variation in texture, and wheats that give evidence of being genetically impure in gluten strength. Such samples are frequently found among the typical hard wheat varieties, especially in some seasons and when grown under humid conditions. Others might be designated as the border line wheats between the semi-hard and hard varieties. On the other hand, the most familiar example of genetic impurity is to be found in the Michigan Amber variety, a semi-hard winter wheat. (See Table IV.)

The wheat breeder will soon come to recognize such wheats by their peculiar behavior. However, since wide variability in "time," to a considerable extent, is coincident with seasonal influences, one should not be guided wholly by the results of one season's trials, but should test such varieties or strains for several seasons.

No difficulty has been experienced in applying the 10% variability rule to tests made on typical soft, semi-hard, and hard varieties, when grown under their accustomed range of adaptability for quality, for their respective classes.

The fermentation cabinet. A medium sized bell jar was employed in the early experiments of Cutler and Worzella (1931), when a suitable technique was being developed for the fermentation test. Being transparent, the bell jar lent itself admirably for this purpose since regular observations could be made in watching the behavior of the dough-balls. However, it was found difficult to maintain proper temperatures of 80° F.[6] since to place in, or take out a beaker, the bell jar had to be lifted.

The accompanying cut illustrates a new fermentation cabinet which was designed by the authors in 1932 and used in the past season's tests. This cabinet combines roominess, shelf space which can be rotated, temperature control, transparency through five surfaces, one of which is a small full-length hinged door, as well as, cheapness. It is unnecessary to have automatic thermostatic control in this cabinet since an operator must be available at all times to make regular observations concerning the behavior of each dough-ball; to remove broken ones and make up and put in new dough-balls.

Experimental

"Time" as a measure of "quality" in wheat. At the present time it is generally recognized that one of the greatest difficulties in breeding improved varieties of wheat is the lack of a simple yet reliable method

[6] Pelshenke (1930) reports that he used somewhat higher temperatures, but considerable study of this question revealed that more uniform results can be expected at the temperature indicated, 80° F. Moreover, this temperature is easily maintained since it is similar to the average room temperature.

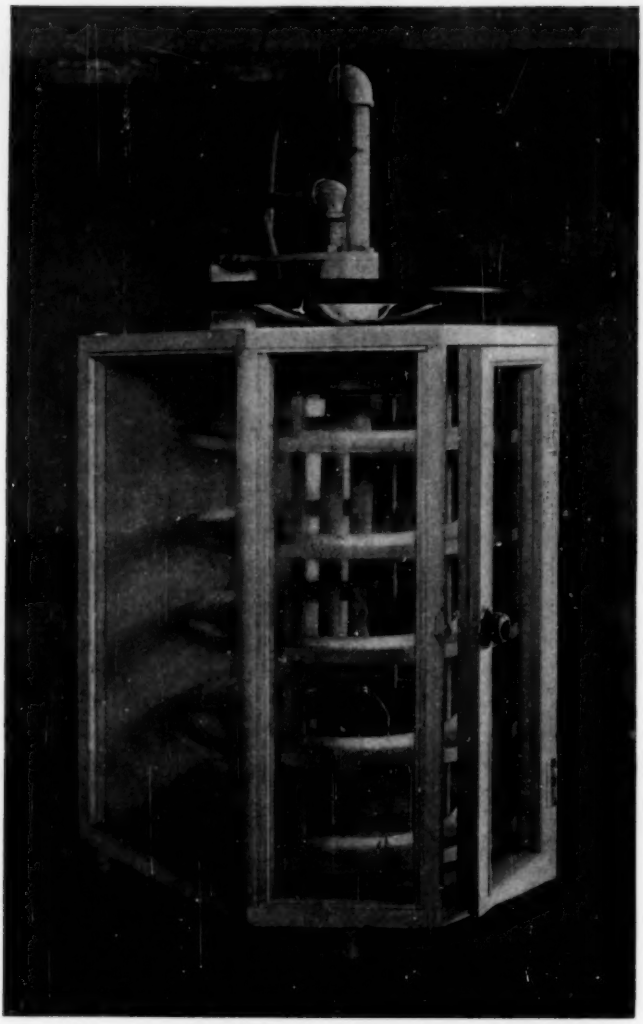

Fig. 1. Fermentation cabinet suitable for testing "quality" of wheat by the wheat-meal fermentation time test.

of estimating milling and baking "quality" when only small quantities of seed are available. Accordingly, if "time," as defined in this paper, is a reliable guide for "quality" in wheat, the fermentation test will meet a long felt need on the part of the wheat breeder and geneticist. Doubtless, too, the miller, the elevator operator, and the cereal chemist will find much use for it. Time and expense in testing for "quality" can thereby be reduced to a minimum and the efficiency of the breeder will be greatly multiplied. He will be able to test the maximum number of wheat selections during the early segregating generations of his

breeding operations and thus quickly and accurately appraise the potentialities of all his hybrids for "quality."

As indicated earlier in this paper, the fermentation test as developed by the authors has been used and studied in a variety of ways during the past four years. Much opportunity has been afforded, therefore, to appraise the reliability of this test in measuring "quality" in wheat. Some of the data accrued were reported, in part, in our original paper (1931), but additional data will be presented here.

The Relation of "Time" to Other Values Used in Evaluating "Quality" in Wheat

In attempting to determine the relation of "time" to other values commonly used in estimating "quality" in wheat, use was made of data secured from the experimental field trials of the standard varieties of winter wheat grown at the outlying stations in Indiana. The data presented in Table I were obtained from several hundred samples representing from 8 to 13 varieties which were tested for protein content (flour), loaf volume, and absorption, through an arrangement with the National Milling Company Laboratory, Toledo, Ohio. The "time" and vitreous kernels were determined by the Agronomy Department at Purdue University Agricultural Experiment Station. Table I gives

TABLE I

CORRELATION BETWEEN "TIME," AS MEASURED BY THE FERMENTATION TEST, AND PROTEIN (FLOUR), LOAF VOLUME, ABSORPTION, AND VITREOUS KERNELS, RESPECTIVELY, OF 8 TO 13 VARIETIES OF WINTER WHEAT GROWN AT 5 TO 9 LOCATIONS WIDELY DISTRIBUTED IN INDIANA. CROPS OF 1929, 1930, 1931, AND 1932

Harvest year	Number of samples	Coefficient of correlation between "time" and			
		Protein (flour)	Loaf volume	Absorption	Vitreous kernels
1929	45	+.60±.152	+.07±.24	+.81±.083	+.74±.107
1930	101	+.49±.142	+.56±.128	+.60±.119	+.48±.143
1931	100	+.82±.062	+.84±.054	+.85±.053	+.84±.057
1932	63	+.70±.088	+.74±.078	+.54±.124	+.55±.121

the correlation coefficients between "time" and other indices used in estimating "quality."

By examining the data year by year, it will be observed that significant positive correlations were obtained, for all years, between "time" and protein content, loaf volume, absorption, and vitreous kernels, respectively, except in the case of loaf volume in 1929. The authors do not attempt to explain the irregularity in results obtained for loaf volume on the 1929 crop. With this exception, however,

there is a very definite trend from season to season. Pelshenke (1930, 1933) using similar technique also reports a close relationship of "time" and loaf volume.

"Quality" in winter wheat varieties as measured by the fermentation test. The data as presented in Table II show the "time" obtained

TABLE II

WHEAT-MEAL FERMENTATION TIME TEST OF "QUALITY" IN WHEAT APPLIED TO 8 VARIETIES OF WINTER WHEAT GROWN AT FROM 5 TO 9 STATIONS IN INDIANA. CROPS OF 1929, 1930, 1931, AND 1932

Variety	Number of tests	Harvest year							
		1929	Ranking	1930	Ranking	1931	Ranking	1932	Ranking
		Min.		*Min.*		*Min.*		*Min.*	
Michikof	13	213	1	299	1	372	1		
Purkof	32	194	2	246	2	283	2	201	2
Michigan Amber	32	163	3	168	3	96	3	71	3
Fultz	29	133	4	146	4	82	4	53	4
Fulhio	25			117	5	72	5	43	6
Trumbull	25	87	6	99	6	70	6	44	5
Gladden	25	81	7	66	7	55	7	39	7
American Banner	29			52	8	37	8	30	8

for 8 varieties of winter wheat grown at from 5 to 9 locations in Indiana over a 4-year period and subjected to the fermentation test. These data serve to emphasize the reliability of this test.

It will be seen that the tendency of varieties to react the same way in different seasons is an outstanding feature of the data in the above table. Throughout this four-year period the ranking of the varieties from year to year is regular and consistent with one exception—Fulhio and Trumbull in 1932. Of importance too is the fact that all varieties responded almost uniformly to the climatic conditions prevailing in 1930 which were favorable to the production of high "quality" wheat. When these data are also considered in the light of the significant correlations as shown in Table I, it becomes very apparent that the time test accurately portrayed the "quality" differences so characteristically inherent in these wheats.

Inter-annual correlations. Inter-annual correlation coefficients were calculated for the purpose of learning the extent to which there was a tendency for hybrid selections and pure line strains of diverse nature to react the same way in different seasons. This may be considered to be a measure of the extent of inheritance under the normal conditions of environment (Zinn, 1920; Hayes, 1928). Simple correlations were made between the results obtained for quality, as expressed in "time," in the harvest seasons of 1931 and 1932, respectively. Two series of

tests are reported as follows: (1) Sixty-seven hybrid selections from F_5 families derived from a cross between two varieties of wheat made to combine suitable milling and baking quality with desirable winter hardiness, and (2) twenty-six pure line strains selected from Michigan Amber as shown in Table III.

TABLE III

INTER-ANNUAL CORRELATION COEFFICIENTS IN SOFT WINTER WHEAT HYBRID SELECTIONS AND PURE LINE STRAINS RESPECTIVELY. CROPS OF 1931 AND 1932

Number of samples	Designation	Coefficient of correlation
67	Hybrid selections	$+.671 \pm .0453$
26	Pure line strains	$+.764 \pm .075$

These coefficients, though limited only to one season's tests, indicate very significant positive correlations of high magnitude. They emphasize that genetic factors play an important part in determining "quality" in wheat, which is apparently correctly measured by the fermentation test. More extensive investigations concerning the mode of inheritance of "quality" are under way at present in the Agronomy Department.

Strains selected for "quality" from standard varieties by the fermentation test indicate genetic differences. The fermentation test seems capable of accurately selecting strains of wheat from standard varieties which vary genetically. This fact is illustrated in the Michigan Amber variety, a semi-hard wheat, which has been grown widely in Indiana for many years. This variety has been very popular among millers though it is recognized as variable in texture and "quality." The strains shown in Table IV represent pedigree selections made on

TABLE IV

MICHIGAN AMBER STRAINS SELECTED FOR "QUALITY" BY THE WHEAT-MEAL FERMENTATION TIME TEST. CROPS OF 1931 AND 1932

Strains	Time	Strains	Time
	Min.		*Min.*
1—2426	315	1—0726	82
1—3226	191	1—1926	80
1—2126	139	1— 626	76
1— 526	115	1—1526	62
1—3326	106	1—2326	66
1—4326	99	1—01426	57

the basis of "quality" by means of the fermentation test taken from the variety test series, from a strain which was derived from a so-called "pure line." Some 12 lines out of many others have been isolated and grown for two years with the following results.

Similar data are available for the Fultz and Rudy varieties which are also well established varieties in the semi-hard wheat districts.

Blending tests lend additional support to the time test. It will be of interest not alone to wheat breeders, but as well to millers and cereal chemists who are interested in blending wheats in flour production, to examine the data obtained when "time" was made the basis for blending. Standard hard and soft wheats were used in these tests. The hard wheats analyzed 14% to 17% protein, and the soft wheats 8.5% to 10.5%. The blends were made on the basis of weight of meal. Nine different combinations of wheats, and 81 separate blends, including in all some 450 single dough-balls, are reported in Table V. The "time" of the straight hard and straight soft wheats, respectively, are shown at the top and bottom of each column.

TABLE V

THE WHEAT-MEAL FERMENTATION TIME TEST USED AS A BASIS FOR BLENDING WHEATS

Proportion of blended wheat	1 min.	2 min.	3 min.	4 min.	Actual average	Expected average	Deviation	
					Min.	*Min.*	*Min.*	*Pct.*
Straight hard	369	324	335	355	346	346		
9 parts hard to 1 soft	340	282	313	361	324	319.4	4.6	1.44
8 parts hard to 2 soft	329	299	308	325	315	292.8	22.2	7.58
7 parts hard to 3 soft	324	231	282	295	283	266.2	16.8	6.31
6 parts hard to 4 soft	374	116	215	291	249	239.6	9.4	3.92
5 parts hard to 5 soft	289	87	170	254	200	213.0	13.0	6.10
4 parts hard to 6 soft	261	84	152	194	173	186.4	13.4	7.19
3 parts hard to 7 soft	234	72	113	148	142	159.8	17.8	11.14
2 parts hard to 8 soft	217	63	104	97	120	133.2	13.2	9.91
1 part hard to 9 soft	157	51	79	65	88	106.6	18.6	17.45
Straight soft	133	52	70	63	80	80		
Average							14.33	7.89

It will be seen that as the amount of meal of the higher or long time high protein wheat is reduced in the blend, the "time" or "quality" of the mixture is correspondingly reduced or vice versa. The results show a significant relationship to mathematical expectancy. While further studies are essential, the data suggest possibilities which the fermentation test offers to the miller for making blends; furthermore, it lends additional support to the validity of this test as a measure of gluten strength in wheat.

The miller and his needs are given due consideration in breeding new wheats. In order to know the "quality" of wheat desired by the miller one of the first steps in our wheat breeding program was to obtain a large number of samples of wheat direct from Indiana millers, who

are using Indiana produced wheats in a large way in the production of flour. This has now been carried out for three years, and will be continued in order to check seasonal variation. All samples thus obtained (some 250 to 500 samples have been collected to date) are subjected to the wheat-meal fermentation time test and the results obtained are used as a guide for the "quality" of wheat desired by our millers. According to the data accruing from such tests during the past three years it seems clear that our Indiana millers require wheats of a peculiar "quality" and these fall into a pretty well defined "time category." This category seems to be delimited by 20 minutes as the lower limit and 185 minutes as the upper limit. Naturally different millers have different demands and these may vary somewhat from season to season. In the main, however, the lower and upper limits remain quite fixed and serve as a guide to the needs of the miller of pastry flours. The bulk of the wheat samples tested appear to fall within the 50 to 125-minute division.

A classification of wheats based on utility and "quality" is suggested. Studies of "quality" with the aid of the fermentation test on a wide range of wheats including classes, varieties, strains, and commercial samples, have served to emphasize the possibility of classifying wheats on a utility basis when "time" is made the measure of "quality." Accordingly, two general groups of wheat might be set up, viz: Soft or pastry wheats and hard or bread wheats, respectively (the durum wheats are not considered in this classification). These, in turn, can each be further subdivided into four or more classes or "time" groups in keeping with gluten strength and uses or purposes to which such wheats are put as follows:

I. Soft or pastry wheats

Classes	*"Time" groups*
Very weak	less than 30 minutes
Weak	30–50 minutes
Medium strong	50–100 minutes
Strong	100–175 minutes

II. Hard or bread wheats

Weak	150–225 minutes
Medium strong	225–300 minutes
Strong	300–400 minutes
Very strong	400 and over

The authors fully appreciate the difficulties envolved in a proposal to set up ironclad groups by which wheats may be classified on the basis of utility and "quality." Obviously, there must of necessity be flexibility to such a classification in order to allow for variations in the behaviour of wheats due to soil and climate influences, not to mention the requirements and methods of the miller. The fact too that there

is an overlapping between the soft and hard wheats, where the strong of the soft and the weak of the hard are similar in milling and baking qualities and may not infrequently be used for the same purpose, seems to add to the difficulties. Notwithstanding all of these variations, with utility and "quality" as the basis, it seems to matter little how the "quality" varies so long as the different manifestations can be correlated with utility. The value of such a scheme of classification lies in the fact that the users of wheat, whether interested in the manufacture of flour for cakes and bread of the different types, for biscuits, pastry, crackers, or flour for family uses, are enabled to find common ground on which to stand with the wheat breeder and producer. The authors offer such a classification for purposes of inviting discussion rather than as a perfected working model.

Flour Granulation as a factor of "Quality" is not overlooked. The soft wheat miller gives due consideration to flour granulation since this characteristic receives much emphasis by the baker of pastry products. In buying wheats, therefore, he follows the general principle that dark, hard, vitreous berries when milled produce granular flours and therefore avoids such wheats as much as possible. While there are some exceptions to this principle, nevertheless, it stands as a working guide to the miller who desires to produce the smooth, non-granular, fine, velvety flours used so much in making pastries. Now, it should be made clear that the fermentation test does not measure flour granulation except in so far as this characteristic is correlated with texture.

The practice followed, therefore, is to regard with suspicion those strains that appear variable in texture and which at the same time record a "time" well up in the upper limits of the time category set up for soft wheats. Such strains are tested for two or more years in order to give them an opportunity to respond to soil and climate variations and reveal a berry that will produce flour too granular for pastry purposes. Generally such wheats are found to run well up toward and even beyond 185 minutes in "time."

Costs of operating the wheat meal fermentation time test. Costs of operating this test are best illustrated by the tests carried out in the Agronomy Department during the seasons of 1931 and 1932. These costs include labor at 30c per hour for grinding the meal and conducting the fermentation tests and the cost of yeast, but do not include the overhead of the grinder and fermentation or warming cabinet, beakers, distilled water, etc. Some 1,760 dough-balls were made at just a trifle under 7c per dough-ball. In 1932 the costs were even slightly reduced.

Summary

The " Wheat-Meal Fermentation Time Test " is the official designation given to a new method for measuring " quality " of wheat with " time " as the index.

A brief description of the method is given. (See also J. Am. Soc. Agron., Vol. 23, No. 12.)

Small amounts of wheat are required in making the test—a minimum of 3.5 gms. has been used satisfactorily.

Tests show significant positive correlations between " time " and protein content (flour), loaf volume, absorption, and vitreous kernels, respectively, on 8 to 13 varieties of winter wheat grown at from 5 to 9 locations in Indiana during each of the last four years.

Tests for " quality " as measured by " time " on winter wheat varieties grown widely in Indiana for four years indicate a regular and consistent response to the fermentation test.

Inter-annual correlation coefficients were computed for " time." Positive correlations of high magnitude are shown between the results of different seasons when wheats of diverse nature were grown under similar environmental conditions.

" Pure line " selections made from standard varieties on the basis of " quality " show genetic differences and these show consistent behavior from season to season when subjected to the wheat-meal fermentation time test.

Blending tests lend support to the validity of the fermentation test and emphasize possibilities which it offers as a basis for blending wheats.

Samples of wheat submitted by the millers are subjected to the fermentation test and the " time " obtained is used as a guide in breeding new wheats.

A classification of wheats based on " time " is suggested for pastry and bread wheats.

Flour granulation in pastry wheats is measured by the fermentation test only in so far as this characteristic is positively correlated with texture of the berry.

The cost of making the wheat-meal fermentation time test is slightly less than 7c per dough-ball.

It is not contended that the fermentation test is the " tell all test," but it should prove a valuable guide to gluten strength when applied with due regard for care and attention to details.

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ERRATUM

REPORT OF THE CEREAL SECTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1932. Volume X, page 162, for lines 4 to 9, inclusive, substitute following method:

To 25 gms. of flour add 241.5 cc. of Sorensen's phosphate mixture of 5.288 pH, at 37° C., and incubate for 1 hour at 37° C., mixing every 15 minutes. Cool the mixture rapidly in a bath of ice water to about 20° C. Then add 7.5 cc. of Na_2WO_4 (15 gms. per 100 cc.), mix well and add 1 cc. of sulfuric acid, mixing continuously. Immediately filter the flour suspension and polarize the filtrate, allowing, preferably, not over a half-hour to elapse between the time of adding the sulfuric acid and the polarization.

To make check determinations, mix 25 gms. of flour with a mixture of 241.5 cc. of water, 7.5 cc. of Na_2WO_4, and 1 cc. of sulfuric acid.

The above quantities of flour sample and solution are such that sufficient filtrate for polarization is obtained in a half-hour or less.

Presentation

of the

Thomas Burr Osborne Medal

to

Clyde H. Bailey

by the

American Association of Cereal Chemists

May 23, 1932

Supplement to Cereal Chemistry
Vol. X, No. 3, May, 1933

Clyde H. Bailey

THE THOMAS BURR OSBORNE MEDAL

The Thomas Burr Osborne Medal is awarded by the American Association of Cereal Chemists for distinguished contributions to cereal chemistry as the result of original research. A recommendation that the American Association of Cereal Chemists recognize notable work in cereal chemistry by the award of a medal, at regular stated intervals, was made by Rowland Clark in his presidential address at the Denver Meeting of 1926.

The Executive Committee, acting on this recommendation, advised that the medal should not be awarded at regular stated intervals, but whenever it seemed appropriate to honor outstanding and distinguished work in cereal chemistry.

A jury or committee on award was appointed by President Clark to name the medal after an American chemist whose work had advanced cereal chemistry, and also to select candidates for the award.

The name of the late Thomas Burr Osborne, a recognized authority on the wheat proteins, was chosen for this honor. Later he was selected to be the first recipient of the medal. The obverse face of the medal shows a portrait of Doctor Osborne in low relief, and on the reverse is the inscription designating the medallist. The first presentation of the medal was made on June 7, 1928 at the University of Minnesota, during the annual convention of the Association which was held in Minneapolis.

C. B. M.

REPORT OF THE THOMAS BURR OSBORNE MEDAL AWARD COMMITTEE

H. E. WEAVER, *Chairman*

As chairman of the Osborne Medal Award, I have the following report to make:

In an early news letter we asked the membership to make suggestions as to candidates for this award. Twelve of our membership availed themselves of this opportunity and all nominated the same man—namely, Dr. Clyde H. Bailey of the University of Minnesota.

On February 17th I submitted the name of Doctor Bailey to the members of the committee along with the recommendations that had been made for him by the membership. Replies have now been received from all members of the committee, all express themselves as favorable to Doctor Bailey's election, therefore, he is unanimously elected to receive the next Osborne Medal granted.

PRESENTATION OF THE MEDAL

After the selection of Doctor Bailey as the recipient of the Osborne medal, the officers of the American Association of Cereal Chemists arranged for the presentation of the medal at the annual meeting of the Association in Detroit, May 23 to 26, 1932. The presentation was made on the evening of May 23, at a joint meeting of the Detroit Section of the American Chemical Society with the American Association of Cereal Chemists. The meeting was opened by Harvey M. Merker, chairman of the Detroit Section of the American Chemical Society, who welcomed the visiting chemists and introduced R. K. Durham, President of the American Association of Cereal Chemists. Mr. Durham then introduced C. B. Morison, who delivered his address: "The contributions of Clyde H. Bailey to cereal chemistry." Following this Mr. Durham made the presentation speech, and Doctor Bailey responded briefly in acceptance of the medal before addressing the audience on the subject, "A quarter century in cereal chemistry."

THE CONTRIBUTIONS OF CLYDE H. BAILEY TO CEREAL CHEMISTRY

C. B. Morison

When the Osborne Medal Award was announced recently, some one asked me what Bailey had been chosen for this honor, as he knew at least four of them, Washington-Bailey, Kansas-Bailey, Connecticut-Bailey, and Minnesota-Bailey, who were well known authorities on cereal and food chemistry. Describing these various Bailey isotropes, I had no difficulty in confirming the identity of Minnesota-Bailey with our friend Clyde H. Bailey.

Curiosity as to just how many Baileys were members of the American Chemical Society prompted me to write to Doctor Parsons for statistics, and I found that twenty-one Baileys were on the rolls of the Society. These included a Fellow of Trinity College, Dublin, Ireland; a Scottish oil chemist in West Lothian, and the rest were Americans in various parts of this country from Harvard University to California.

Minnesota-Bailey, or Dr. Clyde H. Bailey, is a native son of Minneapolis; born in Minneapolis, April 15, 1887, the son of George W. and Sophie McKenney Bailey, and Clyde H. has been a loyal Minnesotan ever since. His family traces back to New England as might be expected, but some of them went out to Minnesota before the Civil War. Doctor Bailey's paternal grandfather came on a few years later and (as I understand the family tradition) sought for bigger and better potatoes than could be found in the state of Maine.

For over twenty years Doctor Bailey has been associated with the state university as a member of what is now known as the Division of Agricultural Biochemistry. He began his career in 1905 as an analyst, or technologist, with the Howard Laboratories of Minneapolis, one of the oldest wheat and flour testing laboratories in this country. Two years later he became connected with the United States Department of Agriculture, Bureau of Plant Industry, as a scientific assistant, and was sent to North Dakota for cooperative work on wheat investigations at the Agricultural Experiment Station. Here, associated with E. F. Ladd, Station Chemist, who later gave up

chemistry to become a "mere" United States Senator, he obtained a start in assembling data and figures which were published in Station bulletins for the benefit of North Dakota agriculture. In 1911 he accepted an appointment to the scientific staff of the Minnesota Agricultural Experiment Station and there began the preparation of more bulletins on wheat studies, which directly have led to his many contributions to modern cereal chemistry, which now may be mentioned without further introduction.

The scientific and technical contributions of Clyde H. Bailey, his students, and coworkers, indicate a wide range of subject interest in the problems of wheat and other cereal grains as they relate to milling and baking. While most of these studies and investigations are concerned with that field, not inaptly termed cereal chemistry, some have important relations to various agronomical factors in wheat breeding and cultivation, and others to the special applications of plant physiology and biochemistry to grain storage problems.

The early work of Bailey originally was published in the form of station bulletins reporting the results of investigations on the milling and baking qualities of wheat flour. Bulletins 89 and 93 published by the North Dakota Agricultural Experiment Station in 1911, were the forerunners of future bulletins from the University of Minnesota station.

The Minnesota wheat investigations include the crop years of 1911, 1912, 1913, and also a study of the milling and baking characteristics of the Marquis type of wheat which was of much current interest at that time. More reports were to be made as chemist to the Minnesota Grain Inspection Department in 1916 and 1917.

During this period the project for a Minnesota State Experimental Flour or Testing Mill was being discussed by those who recognized the need for more extensive and improved facilities for wheat milling and related investigations. Doctor Bailey was deeply interested in this project, and devoted much time and thought to the many technical and other details that should be considered in the construction, equipment and organization of the proposed mill.

The State Testing Mill was authorized by the Legislature in 1919, and finally completed after some delay in 1921. Suitable facilities were now possible for investigations which could be undertaken under comparatively large scale milling conditions and in close contact with laboratory control.

Naturally, Doctor Bailey was appointed as the first director of the State Testing Mill, and his annual reports for the Minnesota crop, years of 1921, 1922, and 1923, present important data not only on the milling and baking values of wheat, but include special investigations made under his direction.

The official report phase of Bailey's work seems to have closed with his resignation as Director of the State Testing Mill in 1924, but it seems pertinent to suggest here that these official bulletins and reports with their repetition of milling and baking tests, flour analyses, accumulation of test data, special investigations and studies of by-

DR. BAILEY AND THE BAILEY FERMENTATION CABINET.

products, had a compelling influence in directing Bailey's work in cereal chemistry toward practical objectives, rather than to more abstract scientific studies of remote applications.

During this official report period of 1911–1924, some of Bailey's work began to appear in various scientific journals. Since Bailey possesses an innate aptitude for laboratory technique and the development of equipment and methods, it is not surprising that in 1916 he communicated a paper on the baking test to the Journal of Industrial and Engineering Chemistry in which he criticised certain objectionable features of the usual procedures employed by most baking technologists. He suggested a better controlled technique, with

mechanical mixing, automatic record of expansion, fixed proofing and automatic temperature regulation during fermentation, which were intended to limit the personal equation in this empirical method. This paper was followed by a second one which discussed the design and construction of a constant temperature fermentation cabinet. In 1930, Bailey again described a further improved design of a fermentation cabinet for use in the baking test.

Bailey's well known studies on the respiration of wheat and other cereal grains with A. M. Gurjar, 1918–1920, and a final paper on shelled corn in 1921 have been of value in their application to the practical problems of bulk grain storage, grading, heating, moisture content, and damage. His study of the respiration of shelled corn was made the basis of a thesis for the doctorate which was conferred on him by the University of Maryland in 1921.

One of Bailey's outstanding characteristics as a cereal chemist is his ability to utilize intelligently and with discrimination the methods, special apparatus, and equipment used in other fields of the biological and physical sciences. As one of his colleagues recently remarked, " Doctor Bailey is always on the lookout for new tools in the field of related sciences which may be applied to his own field of investigations."

Bailey's work on flour grades 1916–1924 with F. A. Collatz, A. C. Peterson, and A. H. Johnson is an early example of his ability to use the methods of physical chemistry in the study of an old problem to the cereal chemist.

Since the ash content of flour is usually taken as an index of flour grade, and a water extract of wheat flour contains inorganic constituents such as the phosphates, it was reasonable to assume that the electrical conductivity of water extracts of flour would increase with the amount of ash present. Bailey and Collatz found that under definite experimental conditions the specific electrical conductivity of the flour-water-extract paralleled the ash content.

A second paper in this series is concerned with the so called " buffer action " of water extracts of flour. This subject is of considerable interest in its relation to the hydrogen-ion concentration of dough fermentation. At the time this paper was written a comparatively small amount of work had been done by American cereal chemists on the influence of the hydrogen-ion concentration (pH) on the problem of flour properties and dough fermentation. Historically, the pH period of cereal chemistry in the United States began several

years after the publication of Jessen-Hansen's work at the Carlsberg Laboratory in 1911. During the late war, the work of Cohn, Henderson, and others, called attention to some of the effects of the hydrogen-ion concentration on flour properties, dough fermentation and the " rope " of bread.

Later, after the war, the relation of the hydrogen-ion concentration to various chemical and biochemical phenomena became more generally recognized and the attention of cereal chemists was again directed to the possibilities of pH as a control factor. As a comparatively new and little understood subject to most milling and bak-

DR. BAILEY AT WORK WITH THE BAILEY HYDROGEN ELECTRODE.

ing chemists, it excited interest and curiosity, especially because it had been proclaimed by some enthusiasts as the royal road to fermentation control and the golden key of flour evaluation for baking.

Bailey and Peterson in 1921 emphasized the " buffer " effect of various grades of flour on the hydrogen-ion concentration, and indicated that high grade flours are buffered less than lower grades, and that apparently phosphates and not proteins exerted the buffer action. Other investigations followed with A. H. Johnson, which gave important data on the specific electrical conductivity, pH and " buffer " action of chlorine bleached flour, and also the influence of time of storage of the flour on these factors.

Bailey's well known study with Sherwood on the change of hydrogen-ion concentration as it develops in dough during fermentation was published in 1923. The work was carried on in a commercial bakery. Observations were made on one thousand pound straights and sponges and the results compared with small laboratory scale doughs. The data obtained is of significant interest, and indicates the relatively small changes in pH over a four-hour fermentation period, and the several variables that are involved in the rate of change in the hydrogen-ion concentration during fermentation. One of the by-products of Bailey's interest in these investigations was the development of a small and convenient hydrogen electrode for use with a small amount (10 cc.) of liquid.

Another study which showed Bailey's interest in the utilization of physical methods in flour testing was the critical investigation of the Chopin extensimeter made with A. M. LeVesconte. The physical properties of doughs are discussed and observations on what the authors term "extensibility" of the dough in the presence of various substances and different conditions as indicated by the extensimeter were reported. The authors concluded that an instrument of this type is of some value in the study of flour baking value.

As a result of the work of Gortner and Sharp, the viscosimeter, one of the chief resources of the colloid investigator, attracted the attention of cereal chemists in its relation to the study of gluten quality. Bailey and J. Hendel used the new viscosimeter procedure in studying the gluten quality of various mill streams.

In 1924, Doctor Bailey obtained leave of absence from the University of Minnesota and organized the research work of the Biscuit and Cracker Manufacturers' Association. He became director of the Research Laboratory and interested himself in the problems of this industry. This marked another period in Bailey's work, and his energies were now concentrated on a comparatively new field in cereal chemistry. An extensive study of cracker-dough fermentation from the physico-chemical side was made with A. H. Johnson and published in 1924.

These few allusions to Bailey's early work have shown their wide range of subject interest, their relation to practical objectives, the development of new methods, and the investigator's ability to utilize the most recent and advanced technique of physical and biological chemistry.

Bailey's work in recent years largely has been published in Cereal Chemistry. He has continued his special studies of hydrogen-ion concentration and among these is one with Emily Grewe (1927) which brought out striking data on the effect of hydrogen-ion concentration on increase of diastatic activity in dough fermentation and the inhibiting effect of hydroxyl-ions.

Extensive studies have been made with Sherwood (1926) on the diastatic activity of flour and its control in relation to the effects of germinated wheat. Another important field of enzyme phenomena in cereal chemistry is that of the proteolytic enzymes. Bailey with A. H. Olsen (1925), A. Cairns (1927) and W. E. Brownlee (1930) has made special studies of yeast, flour, and bread doughs in this connection. The results of the latter study appear to indicate that pronounced changes in the chemical and physical properties of bread doughs are probably not to be attributed to the effects of proteolysis.

The recent work of Bailey with C. G. Ferrari on the carotinoid pigments of flour, the determination of carotin, and relations to storage and various bleaching agents is distinguished for the application of recent methods of spectrophotometry to the study of the problems involved and represent a recognized advance in the fundamental knowledge of this field of cereal chemistry.

Recent work on methods includes a study of dough plasticity by the work input in watt hours of a laboratory mixer (1930), of loaf volume (1930), a study of color in baked products by spectrophotometry, with E. L. Stephens and A. M. Child (1928), and several reports on the baking test investigations of this Association.

Cooperation in agronomical studies are shown by papers on effect of delayed harvesting on wheat quality, with A. F. Bracken of Utah Agricultural Experiment Station (1928); correlation studies of wheat strains in relation to milling and baking qualities, with H. K. Hayes and F. R. Immer of the University of Minnesota (1929); and the water imbibition of frosted wheat, with E. G. Bayfield (1930); and on dockage assessments, with A. E. Treloar, et al. (1931).

Further work on biscuit and cracker problems includes the "checking" of biscuits, with J. A. Dunn (1928); and a study of rancidity, with H. O. Triebold (1932).

Valuable applications of the mathematical analysis of data have been applied in Bailey's study of the relation of protein content of loaf volume, with Sherwood (1926); the correlation of ash content

of wheat and flour, with Sherwood (1928); and correlation of loaf volume, ash, protein, diastatic activity, and moisture to crumb texture and color, with A. E. Treloar and R. C. Sherwood (1932).

Among the various subjects which have interested Bailey recently are the acidity of stored flour, foreign methods for determination of acidity, effects of over-grinding on dough fermentation, methods for determining the "staling" of bread, the proteolytic enzymes of malt, milk in bread making, and the organic phosphorus of wheat.

The allusions which have been made here to some of the various contributions of Clyde H. Bailey to cereal chemistry and related fields are obviously inadequate. No attempt has been made to mention every paper or rate its comparative importance.

This wide range of subject interest is a characteristic of Bailey's work. A recent college textbook was entitled "Pandemic" by its author. The cereal chemistry of Bailey is well described by this term. One line of research has suggested another, and many paths have been opened for further and future exploration. Bailey has been a persistent investigator for over twenty years. Beginning as a cerealist and baking technologist, he knows laboratory routine and the requirements of this type of work. It was inevitable that he should become an investigator. Laboratory routine and the accumulation of results in the form of bulletins did not kill his research interest.

He has been in intimate contact with the problems of agronomy, plant physiology, milling, baking, yeast manufacture and the dairy industry. His investigations largely have had for their purpose a better understanding of fundamentals in the control of practice. He looks forward to improved production control and other practical objectives required by industry, and sees a time when the cereal chemist will be more generally recognized as one of the most important factors in making this possible.

Bailey's work has shown the cereal chemist the value of applying the technique of physical and bio-chemistry to cereal chemistry. His emphasis of the mathematical analysis and correlation of data, which has been of such value in plant and animal breeding and which is now gradually assuming importance in chemistry, is of the highest importance for the progress of research in cereal chemistry. Statements made on insufficient data as the result of a small number of tests, or on a large amount of uncorrelated data have been one of the pitfalls of the cereal chemist.

As a teacher, Bailey has given instruction to many undergraduates at the University of Minnesota, and acted as advisor to graduate students. Sixteen of Bailey's graduate students have been granted the Master's degree, and fifteen the Doctor's degree by his University. His work has been recognized nationally and internationally. He is in constant touch with the work of foreign investigators in both cereal chemistry and agronomy. Future histories of cereal chemistry will probably refer to his work and that of his students as the " Bailey School at Minnesota."

Largely through his efforts, those engaged in cereal chemistry have merged their interests in one organization, the American Association of Cereal Chemists. Active in the foundation of CEREAL CHEMISTRY, our own journal, he became its first editor and has seen it become recognized as a foremost publication in this field.

Bailey's work has been carried on against the pressure of teaching and many other activities which demanded time and energy. He has been prominent in the agricultural and food division of the American Chemical Society, has served the Association of Official Agricultural Chemists as referee on cereal foods, methods for the determination of hydrogen-ions, and on methods of analysis of flours used in foreign countries. He has been Chairman of Committee on Standardization of the Baking Test of this Association, and edited CEREAL CHEMISTRY from 1924–1931.

In the midst of these many activities he has found time to write " The Chemistry of Wheat Flour," which has become recognized as a standard work of reference in this field, with a second edition now in prospect.

Great personal industry has always characterized Bailey's work in cereal chemistry, but it is interesting to know that he sometimes finds time to see a ball game, listen to good music, and read a book on something other than science.

The progress and welfare of his students and friends are not forgotten, and you may feel sure of a hearty welcome whenever you visit his laboratory.

As the second recipient of the Thomas Burr Osborne Medal for distinguished and meritorious contributions to cereal chemistry, Clyde H. Bailey well deserves this honor from our Association, for like the great chemist for whom the medal is named, he has found no substitute for zealous and devoted work, in his chosen field of scientific endeavor.

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1931 **Colloid Aspects of Food Chemistry and Technology,** by William Clayton. Cereal Chem. **9**: 172–173.

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1918 **Some Possibilities of the American Institute of Baking.** Minn. Bakers Service Bull. **1**: 2–4. Reprinted in Northwestern Miller, Dec. 25, 1918, p. 1128; and Bakers Helper, January, 1919.
1919 **Short Courses Aid Better Baking.** Bakers Helper **32**: 482.
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ADDRESS OF THE PRESIDENT OF THE AMERICAN ASSOCIATION OF CEREAL CHEMISTS AT THE PRESENTATION OF THE THOMAS BURR OSBORNE MEDAL, MAY 23, 1932

ROY K. DURHAM

At the 1926 meeting of the American Association of Cereal Chemists in Denver, President Rowland J. Clark proposed that an award of some sort be made to the scientist presenting the most outstanding paper at each annual meeting. Later this was modified to include the most meritorious contribution made to cereal chemistry during each year. The matter finally was referred to the Executive Committee, who proposed that a medal be awarded to outstanding leaders in the field of cereal chemistry only, and that awards should not be made so often as to cheapen the honor that the medal confers.

The first award was to Thomas Burr Osborne, in 1928, for his classic studies in the field of plant proteins, particularly the proteins of the cereals. The medal was named in his honor.

Now, four years later, we are gathered to witness the second awarding of the Thomas Burr Osborne Medal. The Committee of Award this year included Harry E. Weaver as Chairman, and C. J. Patterson, C. O. Swanson, M. A. Gray, and C. L. Alsberg.

Cognizant of his many contributions to the literature of cereal chemistry, his outstanding leadership, his work as a teacher, and his conception and efforts leading to the establishment and successful growth of the scientific journal CEREAL CHEMISTRY, the committee has unanimously elected Dr. Clyde H. Bailey to receive the award.

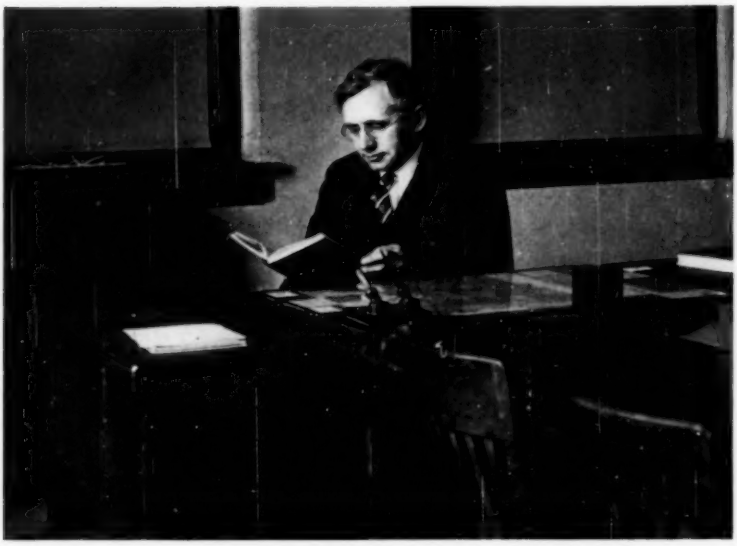
Doctor Bailey is Professor of Agricultural Biochemistry at the University of Minnesota, and cereal chemist in charge of the Section of Cereal Chemistry, Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station.

The recognition given to Doctor Osborne's contributions to cereal chemistry came some twenty years after completion of the cited research and as his active life was closing. In fact even then his ill health robbed him of the pleasure of being present to receive the

plaudits and he was forced to designate a proxy to represent him at the presentation ceremony.

It, therefore, greatly pleases me to have the privilege of presenting the Thomas Burr Osborne Medal to one who is in the prime of life and whose prolific work will be continued for many future years.

Doctor Bailey, will you please come forward. In behalf of the American Association of Cereal Chemists, it is my sincere pleasure to present to you this Thomas Burr Osborne gold medal.



DR. BAILEY AT HIS DESK.

ACCEPTANCE OF THE MEDAL BY DOCTOR BAILEY

Mr. President, Mr. Morison, and Fellow Chemists:

As I endeavor to appraise all the circumstances leading up to this event, it becomes clearly apparent that those responsible for this award, consciously or otherwise, are recognizing the achievements of many individuals. It has been my good fortune to enjoy splendid associations during many years. My professional experiences have been happy ones, and I have been allowed many privileges. Not the least of these has been the sustained contact with competent co-workers. Certain of these have been regular members of various technical, scientific, and teaching staffs; others have been termed "graduate students," although in reality they have been scientific associates in every sense of the term. This shining disk of metal would be divided into many parts if it were fairly apportioned among those whose professional accomplishments have been inseparably interwoven with mine. For me to fail to call attention to their just due would involve unwarranted assumptions.

Mr. President, I am, indeed, deeply appreciative of this award. Your kindly interest in it during your term of office and your expressions on this occasion add to the pleasure that it affords. Mr. Morison, it is not often that one has so capable an advocate as has been afforded me this evening. To the Committee on Awards, and to my fellow chemists who interested themselves in this connection, I wish to express my sincere thanks for their generous consideration and their good sportsmanship.

So I can say with Johann Joachim Becker, chemist-philosopher of the 17th century, "The chymists are a strange class of mortals impelled by an almost insane impulse to seek their pleasure among smoke and vapor, soot and flame, poisons and poverty, yet among all these evils I seem to live so sweetly that may I die if I would change places with the Persian King."

A QUARTER CENTURY IN CEREAL CHEMISTRY

CLYDE H. BAILEY

Somewhat more than a quarter of a century ago a young man of the age of a college freshman applied for a position as laboratory assistant in a commercial cereal laboratory located in a certain mid-western city. To his delight he was given the position and at once started what turned out to be his life work. Such is the force of small events. Oddly enough, the first laboratory manipulation that he was called upon to perform involved a fractionation of flour proteins after a scheme based upon the researches of T. B. Osborne. From this as a beginning, the scope of his activities was broadened until, at the end of four months of service, he suddenly found himself literally the chief chemist and bottle washer, in sole command of the entire laboratory for a brief period. It is probably no exaggeration to assert that at that time he honestly felt that he knew all that was known, if not all that ever would be known of flour chemistry.

Fortunately, these freshman hallucinations, like measles, take a certain brief though violent course and then give place to a more normal condition. New problems crying for solution taxed both knowledge and capabilities to the point where the need for additional training became apparent. The course of this training process is of no interest to you, save that it has continued to the present time and has involved associations prized beyond measure.

When it began twenty-five years ago, certain new developments were appearing in applied biochemistry. That phase of science was just emerging from the domination of organic chemistry and was attempting to make use of new discoveries and hypotheses in the field of physical chemistry. Cereal chemistry was caught up in this major movement. The term "colloid" was occurring with increasing frequency in the literature. To T. B. Wood of Cambridge University, belongs the credit for first calling attention to the colloidal conditions of gluten. With his distinguished colleague, Dr. W. B. Hardy, he developed the significant fact that gluten freed from electrolytes possessed neither coherence nor elasticity, but that minute quantities of certain ions were exceedingly effective in conferring

these properties. Wood suggested an hypothesis to account for variations in the strength of flour, namely, that the electrolytes deposited with the gluten in the developing wheat kernel were responsible for the observed differences in the contribution of the gluten to flour strength and baking quality. While further experience has not wholly supported the practicability of this method of appraisal, it is safe to say that the work of Wood and Hardy stimulated more thought and research in this field than any other single contribution of this period. In the annals of cereal chemistry, it merits a position with the classical contributions.

Colloidal properties of tissues and tissue fluids also interested physiological chemists, and the next sequence of events involved the introduction of Martin Fischer of Cincinnati, and his studies of edema. Certain essentials of his technique in studying the swelling of proteins as a function of the ionic content of solutions in which they were immersed was borrowed by Upson and Calvin of Nebraska, and applied to gluten studies. Their work in turn was elaborated upon by Doherty and Gortner of Minnesota, who concluded that the contribution to flour strength made by the gluten was a function of the rate of imbibition of water in the presence of a certain concentration of weak acids (1919–1920).

About this time Wolfgang Ostwald and his associate, Heinrich Lüers, published a series of papers in which the entire subject of the colloidal structure of dough and its constituents was discussed at some length. This was a monumental contribution and stimulated much useful thinking. Cereal chemists, particularly in America, were not to be diverted from one of their major objectives, however, and sustained efforts were made to utilize measurable colloidal properties in the actual estimation of baking quality. Sharp and Gortner's paper dealing with the relation of viscosity of acidulated flour suspension to baking quality represents one of the milestones of progress. Certain significant differences between flour proteins emerged from their studies, and it appeared that glutenin was chiefly responsible for the differences in viscosity that were observed. The rate of change in viscosity per unit change in concentration of the viscous material—the quality constant "b"—was seemingly related to baking quality. Quantitative measurements of the viscosity of such leached and acidulated flour suspensions are not at all simple to make, as has been demonstrated by the painstaking study of the method conducted by Arnold H. Johnson. This has made it difficult of conversion into

a regular laboratory method. Again the agreement between such observations and the results of baking tests has not always been of a high order, and it is often impossible to determine whether it is the viscosity value or the baking test that is at fault. It is my candid opinion that many of the properties of bread attributed to gluten characteristics may have little to do with the gluten itself. In the study of gluten "quality," the greatest care must be taken to insure that other variables are brought under control, something that is not always simple and easy of accomplishment.

This caution deserves special emphasis in view of Working's observations upon the lipoids. It is possible that relatively small proportions of these substances may serve to exert a large effect upon certain important colloidal properties of gluten and of dough. I strongly suspect that many of the assumed differences in the "quality" of gluten may have been occasioned by the qualitative and quantitative differences in the impurities mixed with the gluten proteins and particularly the lipoids. The latter merit more attention than they have heretofore received.

Following the extensive and painstaking researches of T. B. Osborne, there was more or less of a lapse in the study of the chemical constitution of cereal proteins. For one thing, the introduction of physico-chemical methods of study was occupying the attention of many of the best workers. Again, I suspect that many chemists lacked the courage to attempt the massive scale of operations then necessary to such studies. With the announcement of Van Slyke's procedure about twenty years ago, a new tool became available to the protein chemist, since the fractionation of the products of protein hydrolysis could be carried out with a relatively small quantity of the purified protein. Blish applied the Van Slyke method to a study of gliadin and glutenin preparations from strong and weak flours. No adequate basis for the distinction of gluten proteins of superior quality was afforded by these studies, and Cross and Swain later confirmed these conclusions.

During the past decade, there has been a renewal of the attack upon the fractionation of flour proteins. D. Breece Jones, with his co-workers in the Department of Agriculture at Washington, has contributed largely to these investigations. He separated glutenin into two fractions designated as α and β glutenin, by precipitation with ammonium sulfate solutions of adjusted concentration. The two fractions differed substantially in the composition of the products

of acid hydrolysis, the β glutenin being lower in amide and arginine nitrogen and higher in cystine and lysine.

Blish has recently employed a method of thermal fractionation of flour proteins which we have employed in our laboratories. Crude gluten dispersed in dilute acetic acid (with a small quantity of potassium sulfate present), to which alcohol is added to hold the gliadin in solution, is cooled to 18–20° C., when the glutenin is precipitated. This is removed and the solution further cooled to 11–12° C. when another protein fraction is precipitated. This fraction, on further purification to render it comparatively free from gliadin, possesses physical properties which distinguish it from the latter. It is being subjected to further study in an effort to determine whether or not there are distinguishable differences in chemical constitution as well.

A thermal fractionation of gliadin was effected by Havgaard and Arnold H. Johnson at the Carlsberg laboratory a few years ago. It thus appears that "gliadin" and "glutenin" may not be the simple proteins postulated by Osborne, but may, indeed, be mixtures of distinct substances with different characteristics and chemical constitution.

In their discussion of the prolamines, Hoffman and Gortner (1925) prepared and named several new prolamines of the cereals, including kafirin, speltin, durumin, dicoccumin, monococcumin, secalin, sativin, teozein, and sorghumin. Kafirin was found to have the unique property of being almost insoluble in 70% ethyl alcohol but very soluble in hot alcohol. Larmour (1928) did the same with the glutelins. Substantial differences in the distribution of nitrogen in the products of hydrolysis were observed even among species of the same genus. Thus 8.1% of the nitrogen appeared in the ammonia fraction in the instance of glutelin of Triticum spelta, and 14.8% in glutenin from T. vulgare. Arginine, nitrogen and total basic nitrogen also varied through a wide range.

The difficulties attendant upon the separation and purification of a globulin of wheat have long been recognized. This has been further emphasized by Gortner, Hoffman, and Sinclair, who stressed the inadequacy of the old concept that a globulin is a protein "insoluble in water but soluble in dilute solutions of salts of strong bases with strong acids." The definition of the solvent laid down here is very broad, and these researches established that solutions coming within the limits of this definition might extract or peptize all the way from 10% to 74% of the proteins present in high grade

or patent flours. With a single flour the relative range of solvent action of the various saline solutions was through at least 500%. It is thus obvious that a re-definition of the flour proteins has become necessary, and that the precise relation of these substances to flour quality has become more, rather than less, intricate of appraisal.

In the older work on flour, the starch was largely disregarded, or at least it was considered to be essentially a diluent of the important constituents. Yet the starch constitutes upwards of three-fourths of the dry substance of average patent flour. The work of Alsberg and his several associates at Stanford University, served to open up the issue of possible variation in the properties of starch itself. Their work suggested that there was an appreciable difference in the physical properties of hydrated starch pastes prepared from starch separated from different species of the wheat group (genus Triticum). Mangels' recent work indicates possible differences within a species. In the light of Samec's work with starch, it is conceivable that these physical differences may be a reflection of differences in the chemical make-up of the starch granule—in the ratio of α amylose to β amylose, or in the constitution of either or both of these components of the granule.

Closely related to these studies of the physico-chemical properties of starch is the interesting hypothesis of Katz relative to the staling of bread. Katz suggests that the heat treatment of raw starch pastes incidental to the baking of bread results in a metastabile form that constitutes the so-called "fresh" state. With the lapse of time, this form undergoes a progressive decrease in its water-imbibing capacity, syneresis is involved and the stale condition results. The fresh state can be restored by re-heating.

The enzymes of flour have received their share of attention in this as in other fields of applied biochemistry. Liebig (1909), Baker and Hulton (1908), and Ford and Guthrie (1908) pioneered the earlier explorations of the enzyme problem and stressed the significance of the maintenance of a suitable level of fermentable sugars in a bread dough. Wood's (1907) contribution lent further emphasis to this phase of flour strength. Without a reasonable activity of the amylase of dough, fermentation of the dough is difficult if not impossible to control and manipulate.

Proteases have received attention as well, and in the writer's laboratory the correlation between flour grade and protease activity has been established. At first thought, these protein-hydrolyzing

enzymes may appear to be undesirable, yet in certain situations they may play a useful role. Thus they may serve to facilitate amylase activity as suggested by several investigators including Ford and Guthrie. A certain moderate protein degradation may actually improve the plastic properties of bread doughs prepared with strong bread flours, while a substantial degradation appears to be essential to the production of soda crackers.

Catalase, which decomposes hydrogen peroxide to molecular oxygen and water, is encountered in a more active form in the germ or embryo tissues and the pericarp or branny covering of the wheat kernel. Its activity is accordingly correlated with the degree of refinement of flour, being at the lowest level in the highest grades of flour.

The pigments of the cereals have been elucidated with an increasing degree of precision as the chemistry of these complex organic compounds has been unraveled. Painfully and by slow degrees, their chemical constitution has been disclosed. Through the work of Zechmeister, Kuhn, Spoehr, Karrer, and their associates, we not only have been provided with a more complete and rational basis for their classification and fractionation, but we also are thus acquiring some definite ideas concerning their actual chemical constitution. It is highly unwise to assume that this record is completed as yet, however.

The carotinoid pigments, and particularly the carotins and xanthophylls, have been of particular interest to the flour chemist because of their contribution to the yellow hue of flour. The refinements of physical instrument construction now make available means for estimating their concentration with fair precision, even when the actual quantity present in a natural flour is of the order of two parts per million. Doctor Ferrari recently stated that he was satisfied that the concentration of carotin in the extracts with which he worked could be determined within the limits of error of $\pm$ 0.02 parts per million.

This is the substratum for the effective action of the common bleaching agents of the flour mill. The cereal chemist has been introduced in chronological sequence, to nitrogen peroxide, chlorine, nitrosyl chloride, nitrogen trichloride, and benzoyl peroxide, for use in this connection, and is familiar with their applications. Many cereal chemists are not fully aware of the extensive and growing list of other reagents advanced for this same purpose. Certain of these are included in the following list, and this list is not represented as

being complete: Chlorine dioxide, calcium oxychloride, fumaryl peroxide, phthalyl peroxide, peroxides of glycerol, cholesterol, arabinose, glucose; peroxides of such acids as malic, moleic, succinic, citric, lactic, tartaric, saccharic, cinnamic; mixed acyl peroxides; peraldehydes, ozonides, perozonides, peroxozonides and polymers; derivatives of ethylene oxide with irradiation, or inorganic activators; nitrosyl sulfuric acid in SiO_2 gel, N_2O_3 (Instanto), products of interaction of an acid halide and aldehyde with an alkali; sprouted legume flour, and others vaguely described.

Certain applications of physical and physicochemical methods to cereal research, appraisal, and plant process control have been most typical of this recent period. I have already mentioned several phases of colloid chemistry that have received particular emphasis. These have been related, in no small measure, to the concentration of certain ions, including hydrogen-ions, in the solutions employed in converting them into gels or sols. A specific instance of this sort appeared in the development of the process for precipitating the proteins from the acid digest resulting from the hydrolysis of crude corn starch in the manufacture of glucose syrup and anhydrous glucose. By adjusting the hydrogen-ion concentration to the iso-electric point of the corn proteins that are present, their removal is facilitated. The iso-electric points of several of the cereal proteins have been determined and in certain instances by several different methods.

Hydrogen-ion concentration has been of interest in other connections as well, notably in its relation to the relative activity of the enzymes of pastes and doughs prepared from cereal flours and meals. It seems probable that the increase in H-ion concentration of fermenting bread dough may contribute to the functioning of amylases, zymase, and possibly the proteases that are involved in the process.

The relative level of ionic concentration that is found in suspensions of wheat flour in water appears to be correlated with the grade of the flour, when the concentration is registered in terms of electrolytic conductivity.

Again the relative electrolytic resistance of the crushed grain, held firmly between cylindrical electrodes, is a function of the moisture content of the grain. Moisture content of grain is also a function of its dielectric constant, and compact portable devices have recently been made available for measuring the magnitude of these

physical constants as a means toward the rapid estimation of moisture content.

Bread doughs are exceedingly complex, from the standpoint of the organic and physicochemical systems involved. This complicates the application of the observations of pure science. A notable instance is afforded in the effort that has been made during the past score of years to develop laboratory and plant processes for improving the quality of flour and bread. Some success has attended these efforts and certain formulas or practices have emerged that have proved useful in flour and bread production. It must be conceded, however, that no little speculation has attended the effort to explain the manner of their action. Perhaps it is sufficient that many of them operate effectively, yet the inquiring mind will never be content until the *modus operandi* is more fully explained.

In this brief summary of the many accomplishments of the past twenty-five years, it has been possible to mention only a few of the events. Perhaps I have not even selected those which are of major importance; certainly many have been omitted. To those whose work merits a mention that it has not received, my apologies are tendered, and limitations of time must account for numerous omissions that are obvious to the specialists in this field.

This decanting of the flask in which the good wine of our researches has been accumulating these years is a somewhat doleful task, since it inevitably brings to light the dregs and sediment representing our failures and the inadequacy of our findings. Perhaps we can be cheered by the promise of the quarter century just ahead. Certainly we are placing in the hands of the active young investigators of the next generation many useful tools that were not available to the last. In training, and in mechanical facilities, they are equipped to deal with those materials that contribute to the turbidity of this wine. Theirs will be an enjoyable, and, we trust, a profitable task in terms of the pleasure of the quest and the satisfaction of accomplishment.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